2019年4月9日

適格消費者団体
特定非営利活動法人消費者ネットおかやま
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書類送付のご案内

貴団体よりいただきましたご質問につきまして、下記の書類をお送りさせていただきますので、ご査収のほどよろしくお願いいたします。

記

■ 回答書　1部
■ 質問②_【要約】 FOOD Style 21, 18(5), 73-76 (2014)　1部
■ 質問④_「サイエンス」掲載論文　1部

以上

回 答 書

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代表取締役　植松　芳治

2019年3月14日付「質問書」につきまして、下記の通り回答いたします。

記

① 出典元の論文（J Int Soc Sports Nutr, 10:48, 1-12 (2013)）を広告表示の根拠として使用しております。

② 出典元の論文の要約（FOOD Style 21, 18(5), 73-76 (2014)）を添付いたします。
当研究論文は、膝関節に何らかの違和感を訴える健常者を対象に、非変性Ⅱ型コラーゲンを含有する食品とプラセボを対照とした120日間のRCT結果を報告したものです。非変性Ⅱ型コラーゲン10mg／日の摂取が対象者の関節の曲げ伸ばし範囲（可動域）を広げる効果（すなわち関節の動く範囲、柔軟性、可動性を改善させる作用）に有用であることが示されております。査読付き論分であり、研究デザインが二重盲検ランダム化コントロール試験（RCT）であるため、エビデンスの質としても不足はないかと存じます。尚、当研究論文を根拠とする表示につきましても、消費者庁に御指導を賜り、避けるべき表現の使用は控えるよう努めております。

③ 論文を根拠としております。

④ 「サイエンス」掲載論文を添付いたします。
号数：Vol.261

⑤ 米国特許番号：第5529786号，第5637321号，第5645851号，第5750144号，第7083820号

以上

質問②資料

健常者における非変性Ⅱ型コラーゲンの効果

吉成織恵[1、2)]　森山浩義[2)]　塩島由晃[1)]

はじめに

2007年に日本整形外科学会が提唱したロコモティブシンドロームとは、運動器(骨・関節・靱帯、脊椎・脊髄、筋肉・腱、末梢神経などの体を支え動かす器官)の障害により要介護となるリスクの高い状態のことをいう。その原因は変形性関節症や慢性関節リウマチなどの運動器自体の疾患と、加齢による筋力低下や反応速度の低下などの運動機能の低下が挙げられる。運動器の障害は歩行障害を介して生活の質(Quality of life : QOL)を著しく損なう懸念があり、すでに超高齢社会に突入した日本における高齢者のQOLの維持増進や自立した健康長寿の延伸のためには、この予防対策は緊急の課題となる。

中でも運動器自体の疾病となる変形性膝関節症は、有症者は800万人、予備軍も含めると2,530万人と推定されている[1)]。その有病率を年代別でみると、40歳以上では男性42.6%、女性62.4%の割合であり、まさしく国民病といえる(図1)。変形性膝関節症は加齢により膝関節の軟骨がすり減り、膝関節機能に支障をきたす疾患であり、膝の痛みや関節水腫、可動域の制限、O脚変形、歩行障害などの症状が現れる。しかし現れる症状や進行度合いは人により異なる。例えば、X線写真で膝関節の変形が認められていても痛みなどの症状がない人、逆に痛みがひどいにもかかわらずX線写真では変形がほとんど見られない人などさまざまである。症状の進行度合いを知る手がかりとして、自覚症状が挙げられる。現在、

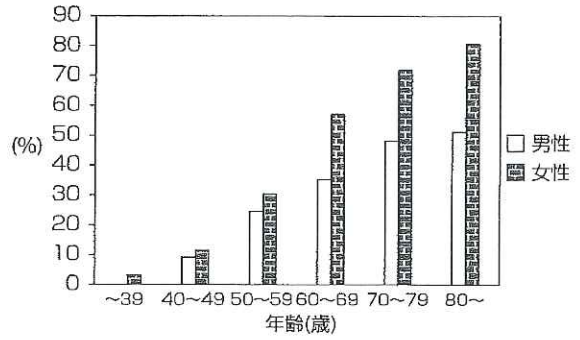

図1　変形性膝関節症の有病率

1) ㈱龍泉堂　2) 帝京大学薬学部

変形性膝関節症と診断されていなくても、違和感などの体の変化に意識を向けることは関節症予防には有効かもしれない。

鶏胸軟骨由来の非変性Ⅱ型コラーゲンを高濃度含むUC-Ⅱ®は、ハーバード大学やヒューストン大学で研究され、これまでに安全性および変形性膝関節症における有効性について動物実験はもとより、臨床試験においても報告されている[2-12)]。この非変性Ⅱ型コラーゲンは、高熱や酸による変性を受けておらずヒト関節中に存在するトリプルヘリックス構造を保ち、活性部位であるエピトープが保持されている。そのためメカニズムの一つとして、経口トレランス(経口免疫寛容)が考えられる[7)]。また、ほかの関節対応素材のように多量摂取を必要とせず、一日当たりUC-Ⅱ®として40mgと少量摂取で効果が期待できる。

今回紹介する臨床試験は、関節症の既往歴はないが、運動により膝に痛みなどの違和感を持つ健常者、いわば関節症の予備軍を対象にしている。

1. 目的

健常者におけるUC-Ⅱ®の有用性について、二重盲検無作為プラセボ対照試験を120日にわたって行った。また同時に試験期間中における健康事象(副作用など)についても検討した[13)]。

2. 臨床試験

2-1　試験デザイン

本試験は二重盲検無作為プラセボ対照試験である。被験者は膝に腫れや痛みなどの関節症の既往歴がなく、高負荷運動(ステップミル)により膝関節に何らかの違和感を持つ健常者55人を対象とした(包含基準と除外基準は表1)。被験者は無作為にプラセボ群(28人)とUC-Ⅱ群(27人)に分け、120日間実験を行った。UC-Ⅱ群には40mgのUC-Ⅱ®(うち10mgは非変性Ⅱ型コラーゲン)のカプセルを、プラセボ群には結晶セルロース、ステアリン酸マグネシウム、二酸化ケイ素を混合したカプセルを一日1粒ずつ摂取してもらった。

表1　包含基準と除外基準

包含基準
・年齢:　30～65歳
・BMI値:　18～35 kg/m^2
・膝関節の健康基準:　休息時に膝関節の違和感がなく、ステップミル(強度レベル4) を10分間以内行うことにより、11点のリッカート尺度において少なくとも5点の膝関節に違和感を持つ者
・試験期間中に食習慣や運動習慣を変えない者
・病歴から健康であると判断された者
・試験内容を理解し、告知に基づく同意に署名した者
・試験期間中に出産制限に同意した女性
除外基準
・NSAIDsの使用者(ただし心臓保護のためのアスピリン使用者は可)
・抗炎症薬やオメガ-3-脂肪酸の常用者(ただし使用後2週間経過している場合は可、また　関節保護のためのサプリメントを常用していても30日経過している場合は可)
・敗血症性関節炎、慢性関節リウマチ、痛風、パジェット病、関節骨折、先端巨大症、血色素沈着症、ウィルソン病、先天性関節疾患、またそれらによる2次的関節症
・AIDs、HIV、強直性脊椎炎、慢性疲労症候群、CREST症候群、クローン病、皮膚筋炎、線維筋痛症、バセドウ病、橋本甲状腺炎、狼瘡、多発性硬化症、重症筋無力症、悪性貧血、壊疽性胆嚢炎、一次胆汁性肝硬変症、乾癬、レイノー症候群、慢性関節リウマチ、サルコイドーシス、強皮症、シェーグレン症、側頭動脈炎、潰瘍性大腸炎、白斑、その他免疫不全や自己免疫疾患
・膝や腰関節の交換手術をした者、6カ月以内に他の外科手術をした、またはその予定の者
・歩行困難者、寝たきりや車椅子の者、身体障害を持つ者
・12カ月以内に免疫抑制を薬使用した者
・3カ月以内にグルココルチコイドやヒアルロン酸を注入した者
・慢性的な痛みがある者
・炎症性腸疾患、頻繁な下痢、体重減少のための手術、胃または腸の切除、胃不全麻痺、乳糖不耐性等の試験の評価に影響すると予想される者
・腎症、肝臓病、内分泌異常、心臓病、肺病、脾臓病、神経症、胆汁障害の者
・ハーブ製品、豆や卵にアレルギーを持つ者
・菜食主義者または絶対菜食主義者
・メラノーマ皮膚癌を除き、2年以内に癌があった者
・1年以内に過剰のアルコールを摂取した者
・30日以内に未認証の薬を摂取した者
・甲状腺機能が低下している者、神経性食欲不振症、過食症や強迫性摂食障害者
・脊髄損傷、多発性硬化症、パーキンソン病を含む神経障害者
・妊娠中、授乳中、または試験中に避妊する意思がない者

2-2　評価方法

本試験の有用性の評価は以下の3項目で行い、ベースライン(0日目)、30、60、90、120日目に測定した。

①膝の可動域範囲測定

②ステップミル上で膝に違和感を持つまでの時間測定

③ステップミルを降りた後の違和感がなくなるまでの時間測定

①は被験者をテーブルの端に座らせ、腰を動かすことなく膝から下を水平に伸ばした際の角度を計測した。②については、被験者がステップミルを登り始めてから、痛みなどの違和感を持つまでの時間を、③はステップミルから降りて違和感が完全になくなるまでの時間を計測した。なお、③の値はベースラインとの差で示した。

3. 結果

各群におけるベースライン時の基本的特徴を表2に示す。二群間での年齢、平均体重、平均身長、BMI値に統計的有意差はない。

表2　プラセボ群とUC-Ⅱ群のベースライン(0日目)

	プラセボ群	UC-Ⅱ群
被験者数(男性/女性)	28 (12/16)	27 (11/16)
年齢	46.6 ± 9.6	46.1 ± 7.7
平均体重 (kg)	77.4 ± 16.6	75.4 ± 14.9
平均身長 (cm)	168 ± 11	167 ± 10
BMI (kg/m2)	27.1 ± 3.8	26.8 ± 4.1

3-1　膝の可動範囲測定

図2に結果を示す。プラセボ群はベースライン(0日目)と比較して試験開始後30、60、90、120日目のいずれも有意な変化は観察されなかった。しかし、UC-Ⅱ群はベースラインと比較して、90、120日目に膝の可動域が増加した(90日目：73.2±1.9°vs.78.8±1.9°[p = 0.045]、120日目：73.2±1.9°vs. 81.0±1.3°[p = 0.002])。また、

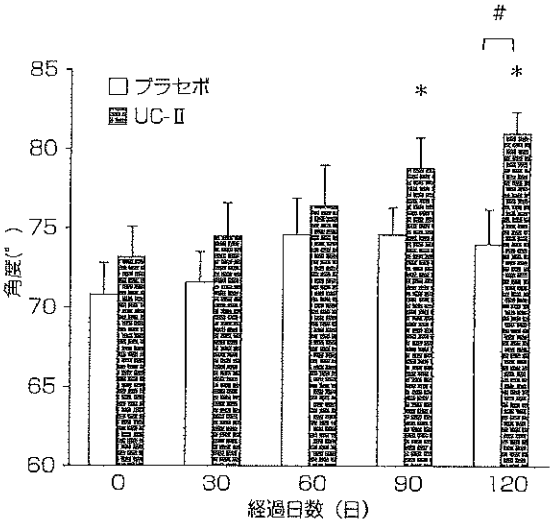

図2　膝の可動範囲測定結果
*，ベースライン(0日目)と有意差あり($p < 0.05$)
#，プラセボ群とUC-Ⅱ群間で有意差あり($p < 0.05$)

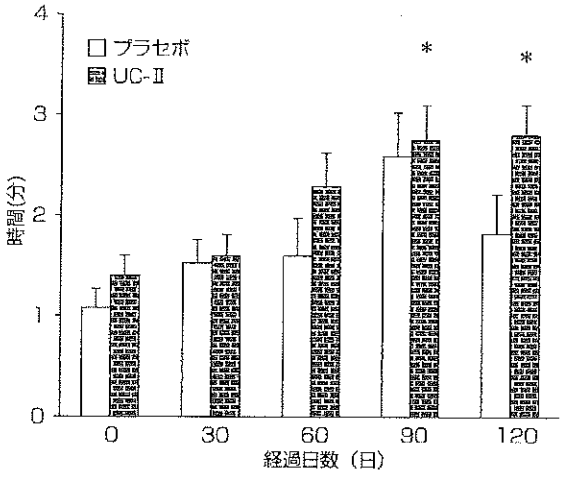

図3　ステップミル上で膝に違和感を持つまでの時間
*，ベースライン(0日目)と有意差あり($p < 0.05$)

表3　ステップミルテストにおいて膝に違和感がなくなった被験者数とその割合

	プラセボ群		UC-Ⅱ群	
	被験者数(人)	割合(%)	被験者数(人)	割合(%)
0日	0	0	0	0
30日	0	0	1	4
60日	0	0	3	13
90日	1	5	3	13
120日	1	5	5	21

120日目のプラセボ群とUC-Ⅱ群の値を比較すると有意にUC-Ⅱ群で増加がみられた(74.0±2.2°vs. 81.0±1.3° [$p = 0.011$])。

3-2　ステップミル上で膝に違和感を持つまでの時間測定

UC-Ⅱ群では、1.4分のベースライン(0日目)と比較して90日目に2.75±0.5分($p = 0.041$)、120日目に2.8±0.5分($p = 0.019$)と関節の痛みなどの違和感が出るまで有意に増加した。一方、プラセボ群では試験期間中での有意差はなかった。また、各測定時のプラセボ群とUC-Ⅱ群の比較においても、有意な変化は見られなかった(図3)。しかし、この結果には運動時に違和感が出ない被験者は除外しており、その被験者数とその割合を経時的に示したものが表3である。UC-Ⅱ群においては高負荷運動下でも違和感が消失した被験者は、60日目には27人中3人(13%)、120日目には5人(21%)と経時的に増加した。一方、プラセボ群ではこのような被験者が90、120日目に1人(5%)だけであった。このような違和感の消失が両群でランダムではなく、UC-Ⅱ群において増加傾向を示すことは重要であり、UC-Ⅱの有用性を推察できるものである。

表4　試験期間中における健康事象のまとめ

	健康事象（器官）	数
プラセボ群	両足首浮腫（筋骨格）	1
	右足首骨折（筋骨格）	1
	副鼻腔炎（耳/鼻/のど）	1
	皮膚感染右足首（皮膚）	1
健康事象数		4
健康事象を報告した被験者数		2/28
UC-Ⅱ群	上気道感染（肺）	3
	食中毒（胃腸）	1
健康事象数		4
健康事象を報告した被験者数		4/27

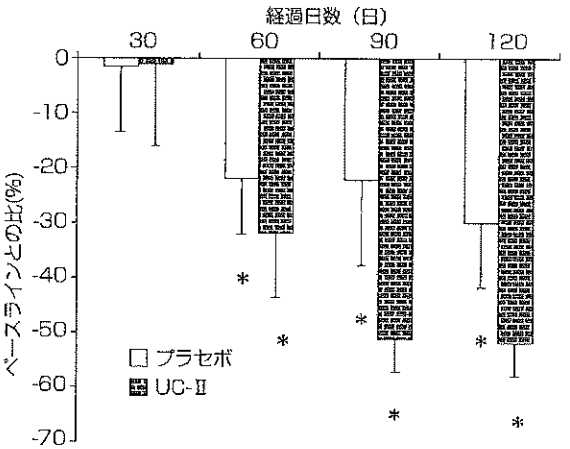

図4　ステップミルを降りてから違和感がなくなるまでの時間
*，ベースライン(0日目)と有意差あり($p < 0.05$)

3-3 ステップミルを降りた後の違和感がなくなるまでの時間測定

関節の違和感が治まるまでの時間は、プラセボ群、UC-Ⅱ群の両群でベースラインと比較して60、90、120日目に有意な減少を示した(図4)。UC-Ⅱ群の方がプラセボ群よりも減少率は大きくみえるが、二群間での有意差はみられなかった。

3-4 安全性評価

今回の試験期間中の健康事象については表4に示した。合計8つの健康事象は両群で等しく観察されたが、どれもプラセボ同様、UC-Ⅱ®摂取に関連するとは考えにくい。またこれらの事象により試験を棄権した被験者がいないことから、UC-Ⅱ®は忍容性が高いといえる。

まとめ

UC-Ⅱは以前から変形性膝関節症や慢性関節リウマチ患者において、痛み軽減や膝の機能改善に有効であるという報告はされていた。しかし今回紹介したように、健常者であっても関節に懸念を抱いている予備軍に対してUC-Ⅱ®が効果を発揮することが示唆された。さらに副作用といえる健康事象が見つからず、忍容性が高いことも確認された。その詳細なメカニズムについては不明な点が多く、今後の検討課題といえる。

おわりに

これまでの報告と合わせて考えると、UC-Ⅱ®は変形性膝関節症などの激しい痛みから、痛みまではいかない違和感まで軽減する可能性が示唆された。しかも一日UC-Ⅱ®40mgの摂取でその機能性が期待できる点は、特記すべきことだろう。超高齢化社会の日本において、UC-Ⅱ®はよりよいQOLを過ごすための抗ロコモティブシンドローム素材であるといえる。

なお、今回紹介した臨床試験の図表は、著作権を持つBioMed Centralに届け出て使用している。

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2001年 工学院大学大学院工学研究科修了、同年 ㈱セイア入社、環境事業担当主任研究員、2003年 ㈱ビオクラフト設立に参画、研究開発部長、同年 ㈱龍泉堂入社、2009年 ㈱マイライフ代表取締役に就任(兼務)、2011年 ㈱龍泉堂代表取締役に就任、現在に至る

質問④資料

Effects of Oral Administration of Type II Collagen on Rheumatoid Arthritis

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Rheumatoid arthritis is an inflammatory synovial disease thought to involve T cells reacting to an antigen within the joint. Type II collagen is the major protein in articular cartilage and is a potential autoantigen in this disease. Oral tolerization to autoantigens suppresses animal models of T cell–mediated autoimmune disease, including two models of rheumatoid arthritis. In this randomized, double-blind trial involving 60 patients with severe, active rheumatoid arthritis, a decrease in the number of swollen joints and tender joints occurred in subjects fed chicken type II collagen for 3 months but not in those that received a placebo. Four patients in the collagen group had complete remission of the disease. No side effects were evident. These data demonstrate clinical efficacy of an oral tolerization approach for rheumatoid arthritis.

Rheumatoid arthritis (RA) is a common chronic illness in which the synovial membrane of multiple joints becomes inflamed, causing damage to cartilage and bone. Although the pathogenetic mechanisms underlying the disease are unknown, rheumatoid arthritis is associated with human lymphocyte antigen (HLA)–DR4 and considered to be an autoimmune disorder in which activated T cells participate (*1*). Type II collagen is a candidate autoantigen for this disease because it is the most abundant structural protein of cartilage, and immunization of animals with the native protein creates arthritis morphologically resembling rheumatoid arthritis (*2, 3*). Patients with the disease have immune responses to native type II collagen (*4*), but whether collagen reactivity participates in the primary pathogenesis of rheumatoid arthritis or reflects tissue degradation is unknown.

Current treatments are inadequate in that they only partially control established rheumatoid arthritis. They also have side effects that limit use early in the disease process and interfere with prolonged administration (*5*). An ideal therapy would decrease inflammation in the joint by a disease-specific mechanism and would lack toxicity. Oral tolerization, a method of inducing antigen-specific tolerance, suppresses animal models of the autoimmune diseases multiple sclerosis, uveitis, and diabetes (*6–11*). In a double-blind pilot trial involving 30 patients with multiple sclerosis, oral administration of bovine myelin antigens decreased the number of T cells that reacted with myelin basic protein (MBP), with no measurable toxicity (*12*). Although favorable trends occurred in the myelin group, clinical efficacy could not be determined because of the small sample size.

Oral administration of native type II collagen ameliorates two animal models of rheumatoid arthritis induced by type II collagen (*13*) or complete Freund's adjuvant (*14*). These experimental findings provided the rationale for a pilot, open-label dose-escalation and safety study in 10 patients with recalcitrant rheumatoid arthritis. Subjects were taken off their immunosuppressive and disease-modifying drugs consisting of methotrexate, 6-mercaptopurine, azathioprine, or auranofin and fed 0.1 mg of solubilized type II collagen daily for 1 month and then switched to 0.5 mg for the next 2 months (*15*). This dose was extrapolated from experiments in the rat adjuvant arthritis model where feeding 3 to 30 μg of collagen attenuated disease (*14*) and the rat experimental autoimmune encephalomyelitis (EAE) model where feeding 500 to 1000 μg of MBP was suppressive (*6, 10*). Six of the 10 patients experienced a substantial clinical response, defined by a ≥50% improvement in both swollen and tender joint counts with two additional disease measures improving by ≥50% [morning stiffness, 15-m walk time, grip strength, Westergren erythrocyte sedimentation rate (ESR), or physician or patient global assessments] and lasting for at least 2 months after the treatment period (*16*). A complete response, that is, disease remission (*17*) with discontinuation of nonsteroidal anti-inflammatory drug (NSAID), occurred in one patient previously on methotrexate and continued for 26 months. There were no adverse effects. Based on the results of this phase I study, a placebo-controlled, phase II trial was undertaken to determine whether clinical efficacy could be demonstrated.

For this phase II trial, 60 patients with severe, active rheumatoid arthritis and who met eligibility criteria (*18*) gave informed consent (*19*) and were entered into the study. They were withdrawn from immunosuppressive drugs if they had been taking them (*20*) and randomized (*21*) to either a treatment identical to that used in the phase I trial (*15*) or an indistinguishable placebo (*22*) to be taken orally for a consecutive 90-day period. Both patients and investigators, except those responsible for medication (*23*), were masked as to treatment. Assessments were performed by the same investigator (D.E.T.) at the initiation of treatment and at 1, 2, and 3 months, generally at the same time of day (*24*).

At the conclusion of the study, 59 of the 60 patients were considered evaluable (*25*); 28 had received collagen and 31 placebo. On entry, demographic, clinical, and laboratory parameters were similar in both groups (Table 1) (*26*). Relative to entry, there was significant ($P < 0.05$) improve-

Table 1. Patient characteristics at entry. There were no differences between groups ($P > 0.10$) detected by either Fisher's exact test or the Wilcoxon rank-sum test (age and disease duration).

Characteristic	Treatment	
	Collagen ($n = 28$)	Placebo ($n = 31$)
Age (years ± SD)	50.3 ± 11.9	55.1 ± 12.9
Sex (% females)	71	68
Disease duration (years ± SD)	9.8 ± 6.2	10.3 ± 8.1
Rheumatoid factor [%, (number tested)]	74 (27)	82 (28)
HLA-DR 4+ [%, (number tested)]	46 (28)	62 (29)
Collagen II antibody (%, titer ≥ 2)	32	13
Prednisone (%, ≤10 mg/day)	25	48
Immunosuppressive* withdrawn (%)	64	58

*Methotrexate, 6-mercaptopurine, azathioprine, hydroxychloroquine, sulfasalazine, auranofin, cyclosporin, cyclophosphamide, or penicillamine. Seven patients were receiving combinations of these drugs (*20*). The remaining patients were not on immunosuppressive drugs at the time of entry because of prior lack of response or toxicity to at least two of the drugs.

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ment in the number of swollen joints, the number of tender or painful joints, joint-swelling and -tenderness indices, and 15-m walk time at months 1, 2, and 3 in the collagen group as compared with placebo patients, except for the number of tender or painful joints at month 2 ($P = 0.06$) (Table 2). Among the collagen patients, the decline in the number of swollen joints, tender joints, and joint-swelling and -tenderness indices were all significant ($P < 0.05$). Four of the collagen patients (14%), as compared with none in the placebo group, had complete resolution of disease (*27*). Table 3 indicates the patients' status by other outcome measures (*16, 21, 28*). Stability or improvement while patients were off immunosuppressives occurred in the collagen group, whereas patients in the placebo group tended to deteriorate. In alternative analyses that reduce the influence of the four placebo patients who withdrew from the trial (*25*), a similar significant ($P \leq 0.05$) improvement from collagen was seen (*29*). A placebo effect resembling that encountered in other RA trials (*30*) was also observed. Four patients (13%) in the placebo group exhibited substantial benefit (*16*) and attained functional class I ranking. This observation reaffirms the critical importance of placebo-controlled evaluations in rheumatoid arthritis. No side effects or significant changes in laboratory values, including rheumatoid factor and antibodies to type II collagen, were noted. There was no evidence of sensitization to collagen, as measured by antibodies to type II collagen. Attempts to assess T cell responses to type II collagen, including release of transforming growth factor–β (TGF-β), were unsuccessful because of the difficulty in demonstrating reactivity to type II collagen in the peripheral blood of RA patients. None of the baseline features, including the presence of collagen antibodies, HLA haplotype, or sex, were associated with responsiveness to collagen (*31*).

This controlled trial provides evidence that oral administration of small quantities of solubilized native heterologous type II collagen is both safe and can improve the clinical manifestations of active rheumatoid arthritis. Baseline values were determined while 64% of the collagen-treated patients were on immunosuppressive drugs (usually methotrexate or 6-mercaptopurine), and further improvement occurred with collagen treatment. If longer term efficacy is established, oral collagen would be a preferable treatment because it is not toxic.

Table 2. Disease variables in collagen-versus placebo-treated patients. Number of patients (collagen/placebo) evaluated at entry = 28/31, 1 month = 27/29, 2 months = 26/26, and 3 months = 28/31; withdrawals were treated as described (*25*); values shown are differences from entry except for patient and physician assessments which are given as percentages. There were no significant differences between groups at entry ($P > 0.05$ for all variables by the Wilcoxon rank-sum test or the χ^2 trend test for patient and physician assessments) (*16*). Comparisons between groups showed significantly more improvement or less worsening in the collagen-treated patients ($P < 0.05$ and $P < 0.01$). Differences between physician assessments in collagen and placebo patients were not significant but showed trends in favor of collagen at 1 month ($P = 0.066$) and 2 months ($P = 0.06$). Qualitatively similar results were found when a two-way analysis of variance was used to adjust for prednisone use. Significant improvement was also observed among collagen-treated patients at 1, 2, and 3 months in terms of the number of swollen joints, the swollen joint index, the number of tender joints, and the tenderness index (Student's t test; all P values are <0.01, except at 3 months, for the number of swollen joints, $P = 0.02$, and the swollen joint index, $P = 0.03$).

Variable	Group	Mean value at entry (± SE)	Difference from entry at month		
			1	2	3
Joints swollen (number)	Collagen	11.8 ± 0.9	−2.7 ± 0.5**	−4.1 ± 1.0*	−3.1 ± 1.1*
	Placebo	12.0 ± 0.8	2.0 ± 1.4	0.9 ± 1.6	1.3 ± 1.4
Joints tender to pressure or painful on passive motion (number)	Collagen	15.8 ± 1.3	−4.1 ± 1.1*	−6.7 ± 1.5	−5.4 ± 1.8*
	Placebo	15.6 ± 0.8	1.1 ± 1.4	−1.1 ± 1.7	−0.1 ± 1.6
Joint-swelling index	Collagen	13.3 ± 1.1	−3.4 ± 0.8**	−4.8 ± 1.2*	−3.1 ± 1.4*
	Placebo	13.2 ± 0.9	2.4 ± 1.8	0.9 ± 1.6	4.3 ± 2.1
Joint-tenderness or pain index	Collagen	17.5 ± 1.3	−5.0 ± 1.2**	−7.6 ± 1.7*	−5.7 ± 2.0*
	Placebo	17.2 ± 1.0	1.6 ± 1.8	−0.5 ± 2.1	3.0 ± 2.4
15-m walk time (s)	Collagen	13.2 ± 0.6	0.0 ± 0.3**	0.25 ± 0.5**	0.5 ± 0.6**
	Placebo	14.9 ± 0.9	1.9 ± 0.6	3.8 ± 1.2	20.8 ± 7.5
Grip strength (mmHg)					
Right	Collagen	105 ± 9	0.1 ± 6.0	6.3 ± 7.8	−0.9 ± 8.5
	Placebo	87 ± 8	−7.3 ± 6.2	−8.3 ± 8.4	−16.4 ± 8.8
Left	Collagen	106 ± 10	0.6 ± 5.6	6.6 ± 7.4*	−0.3 ± 8.8
	Placebo	95 ± 8	−8.9 ± 5.8	−9.3 ± 10.1	−13.8 ± 9.7
Morning stiffness duration (min)	Collagen	155 ± 51	64.8 ± 106	51.2 ± 100	56.4 ± 92
	Placebo	210 ± 55	130 ± 76	168 ± 108	195 ± 100
Patient assessment (%)					
Absent or mild	Collagen	21	41	23*	36*
Moderate	Collagen	54	33	46*	25*
Severe or very severe	Collagen	25	26	31*	39*
Absent or mild	Placebo	16	21	15	19
Moderate	Placebo	35	31	23	10
Severe or very severe	Placebo	48	48	62	71
Physician assessment (%)					
Absent or mild	Collagen	18	41	35	32
Moderate	Collagen	46	33	38	29
Severe or very severe	Collagen	36	26	27	39
Absent or mild	Placebo	6	21	27	19
Moderate	Placebo	42	31	12	13
Severe or very severe	Placebo	52	48	62	68
ESR (mm/hour)	Collagen	39 ± 6	5.1 ± 2.9	4.9 ± 2.8	1.7 ± 3.9
	Placebo	34 ± 5	9.8 ± 5.0	7.8 ± 5.6	3.2 ± 2.8

*$P < 0.05$. **$P < 0.01$.

Although it is possible that the disease could be exacerbated or an allergy to the oral antigen could develop, this was not observed in our study, in animals (*6–11, 13, 14*), in multiple sclerosis patients given oral myelin for as long as 3 years (*12*), or in uveitis patients treated with retinal S-antigen (*32*). All patients in the phase II trial and open-label trial had collagen discontinued after 3 months. Four patients in the pilot study who improved while on collagen experienced a relapse about 3 months after cessation of therapy followed by benefit with reinitiation of collagen. In animals, protective effects of oral tolerance appear to last for 2 to 3 months after termination of antigen feeding (*6*). Recrudescence of disease after discontinuation of oral toleragen has also occurred in multiple sclerosis (*12*) and uveitis (*32*) patients. It therefore appears that additional administration is required to maintain the clinical effects of oral tolerance.

On the basis of studies of oral tolerance in animals, two immunologic mechanisms could explain the clinical response to collagen observed in this study. Feeding type II collagen in RA cases may both anergize $CD4^+$ type II collagen autoreactive cells and generate major histocompatibility complex (MHC) class I– or class II–restricted regulatory cells that sequester within joint tissues and release cytokines that inactivate autoaggressive cells. In animals, feeding large doses of antigen favors T cell anergy, whereas multiple small doses favors the induction of regulatory T cells (*33*). In the EAE model, feeding low doses of MBP activates MBP-specific regulatory cells in gut lymphoid tissue (*10*). These cells are predominantly $CD8^+$ and suppress EAE by trafficking to the central nervous system and releasing anti-inflammatory cytokines, such as TGF-β and interleukin-4, when they encounter MBP presented by MHC molecules in inflamed brain tissue. This process, termed antigen-driven bystander suppression (*10*), implies that an orally administered protein can down-regulate organ-specific autoimmune disease as long as it is a constituent of the target tissue and is capable of inducing regulatory T cells. It is not obligatory for the protein to have the disease-inciting epitopes. Examples of bystander suppression include inhibition of proteolipid protein (PLP)–induced EAE by orally administered MBP (*34*), delay of diabetes in the non-obese diabetic mouse by oral insulin (*11*), and abrogation of adjuvant arthritis by oral collagen (*14*). In all three models, autoimmunity to the toleragen does not appear to initiate disease. Accordingly, our data do not determine whether type II collagen is the primary autoantigen in rheumatoid arthritis.

Although initial clinical efficacy of oral collagen has been shown, questions concerning optimum dosing and long-term control of disease remain. Nonetheless, this study demonstrates the therapeutic efficacy of oral tolerance for a human autoimmune disease and provides the foundation for the development of oral collagen as an easily administered nontoxic treatment for rheumatoid arthritis.

Table 3. Outcome measures in collagen- versus placebo-treated patients. Values are percentages of 28 collagen and 31 placebo patients.

Variable	Entry		Three months	
	Collagen	Placebo	Collagen	Placebo
Worsening status*			7*	35
Analgesic use†			14†	39
Functional class‡				
I	0	0	18	13
II	57	58	39	19
III	43	42	39	58
IV	0	0	4	10

*Represents an increase of 30% or more from the entry value for the joint-swelling index and the joint-tenderness or pain index (*16*). Comparison between groups showed significantly more deterioration in the placebo-treated patients ($P < 0.01$ by the Fisher's exact test). †Narcotic without anti-inflammatory properties, usually acetaminophen with codeine, propoxyphene, or pentazocine, prescribed at any time by the clinical investigator in an attempt to retain flaring patients in the trial. Comparison between groups showed significantly greater numbers of placebo-treated patients requiring narcotics ($P < 0.04$ by the Fisher's exact test). ‡Determined by American Rheumatism Association criteria for functional class (*28*): I, no limitation from arthritis; II, mildly restricted; III, markedly restricted; and IV, incapacity causing virtual bed or wheelchair existence. Trend for improvement in the collagen group not significant ($P = 0.10$ by the χ^2 trend test).

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15. Native type II collagen, isolated from sternal cartilage of chicks rendered lathyritic by administration of β-aminoproprionitrile (*2*), was used to treat the first five subjects in the phase I pilot study. Subsequent patients in the pilot trial and in the double-blind study received type II collagen purified from nonlathyritic chicken sternal cartilage by the identical technique (*2*) and obtained from Genzyme (Boston, MA). Preparations were analyzed for purity by standard biochemical methods (*2, 35*) and tested for arthritogenicity and toxicity in rats (*2*) with findings of batch-to-batch equivalency. Collagen was stored in a lyophilized state (*2*) at −20°C with desiccant. The protein was solubilized in 0.1 M acetic acid for ~12 hours at 4°C, sterilized by membrane filtration, and aliquoted into individual 1.0-ml doses in sterile tubes. Tubes sufficient for ~2 weeks of treatment were delivered on ice to patients and maintained under refrigeration until use. For oral administration, the 1.0-ml aliquot was added to 4 to 6 ounces (118 to 177 ml) of cold orange juice and the mixture drunk immediately. Orange juice provided an additional acid vehicle to inhibit precipitation of collagen and masked the taste of acetic acid. All dosing occurred in the morning on an empty stomach at least 20 min before breakfast or ingestion of other fluids. Smoking was not permitted during this interval.
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18. The following requirements determined eligibility: (i) American Rheumatism Association (ARA) criteria for classic or definite rheumatoid arthritis (*16*); (ii) onset of the disease at age 16 or older; (iii) age at least 18 years; (iv) ARA functional class II or III (*28*); (v) signs or symptoms of synovitis unresponsive to at least one immunosuppressive (Table 1); and (vi) severe active disease defined by at least three of the following: ≥nine painful or tender joints, ≥six swollen joints, ≥45 min of morning stiffness, or ≥28 mm/hour ESR. Exclusion criteria included a degree of structural joint damage not amenable to physical rehabilitation if inflammation subsided after treatment or a serious concurrent medical problem. Some patients (n = 39) represented referrals for treatment of refractory disease by rheumatologists outside Boston; others (n = 10) had received experimental therapy for rheumatoid arthritis in the past.
19. The study was approved by the Beth Israel Hospital Committee on Clinical Investigations and conducted under an investigator-initiated Investigational New Drug (IND) permit from the U.S. Food and Drug Administration.
20. Because of the possibility that patients would receive ineffective therapy or a placebo, study medication was begun immediately after the patient discontinued immunosuppressive drugs (Table 1); patients receiving parenteral gold were not entered because prolonged carryover effects could influence the outcome. Patients remained on their NSAID, prednisone dose (≤10 mg/day), or both, during the 3-month treatment period. NSAID substitution, increases in NSAID or prednisone dose, or initiation of any other antirheumatic therapy with the exception of analgesic agents and intraarticular steroids represented protocol violations. If applicable, patients were requested to practice contraception.

21. A biostatistician (E.J.O.) randomized each patient to either the active or placebo treatment group in blocks of six, stratified by functional class (*28*) severity.
22. The placebo consisted of 1.0-ml doses of 0.1 M acetic acid subjected to membrane filtration.
23. Three investigators (D.C., C.L., and K.L.S.) obtained the randomization and prepared medication but did not have access to clinical data. No unblinding occurred.
24. Conventional instruments were used to measure RA activity (*16*). Assistive devices were permitted for walk times. The clinical investigator cared for the patients during the trial and was responsible for safety monitoring. Laboratory safety assessment was performed immediately before randomization and at 2, 4, 8, and 12 weeks thereafter. The assessment comprised a complete blood count, differential and platelet count, liver and renal function tests, prothrombin and partial thromboplastin times, urinalysis, and ESR. HLA typing was performed for alleles of the A, B, C, and DR/DQ loci (*36*). Serum immunoglobulin M (IgM) rheumatoid factor titers were determined by nephelometry and IgG antibody titers to native type II collagen (expressed as $-\log_2$) by enzyme-linked immunosorbent assay (*37*) immediately before and at the end of collagen or placebo administration.
25. Before unblinding, decisions were made concerning the analysis of five subjects (8%) that failed to complete the study. One was noncompliant and withdrew for personal reasons on day 40 after only a baseline examination. This patient was excluded from analysis and had been randomized to collagen. Four discontinued their study medication before the end of the 3-month treatment because of worsening arthritis. They were assigned the worst score in the sample for the remainder of the study and included in the analyses. All four had been randomized to placebo. One protocol violation occurred with a patient who increased the daily dose of prednisone from 5 mg to 10 mg just before month 2. Because the patient continued to do poorly and the 10-mg dose was consistent with eligibility requirements, the patient was included in the analyses; the patient had been randomized to collagen. No steroid injections or other problems with compliance occurred.
26. Comparisons between collagen- and placebo-treated patients were performed with the Wilcoxon rank-sum test for continuous measures (such as the number of swollen joints), the Fisher's exact test for dichotomous measures (such as narcotic usage), and the χ^2 trend test for functional class and patient and physician assessments. All measured end points such as the number of swollen joints were compared with entry values before testing; qualitative measures, such as patient and physician assessments and functional class, are presented and analyzed without adjustment for baseline responses. The Student's paired *t* test was used to assess whether changes in the collagen group represented significant improvements over baseline values. Reported *P* values are two-sided.
27. Complete resolution is a more rigorous extension of RA remission criteria (*17*), preformulated because of the magnitude of improvement in some patients in the initial trial, and is defined by the following conditions: no swollen or tender joints, no morning stiffness or afternoon fatigue, absent arthritis on physician and patient appraisals, functional class I status, and normal ESR (<28 mm/hour) while off prednisone.
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29. Rather than assigning the placebo patients who withdrew from the trial the worst observed value (*25*), they were given the value from their last visit. Because one of the four dropped out before the 1-month follow-up, that patient was removed from all analyses, reducing the sample size to 28 collagen and 30 placebo patients. By this analysis, the number of tender joints, joint-tenderness index, walk time, patient assessment of severe or very severe disease, and analgesic use was significantly ($P \leq 0.05$) improved in the collagen group compared with the placebo group.
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38. Supported by NIH grants MO1 RR01032 and AG00294 and by a grant from AutoImmune Inc. We thank E. Milford and C. B. Carpenter for HLA typing. In accordance with disclosure guidelines of the Harvard Medical School, D.A.H. and H.L.W. have a financial interest in AutoImmune Inc.

9 June 1993; accepted 23 August 1993

Tyrosine Phosphorylation of DNA Binding Proteins by Multiple Cytokines

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Interferon-α (IFN-α) and IFN-γ regulate gene expression by tyrosine phosphorylation of several transcription factors that have the 91-kilodalton (p91) protein of interferon-stimulated gene factor–3 (ISGF-3) as a common component. Interferon-activated protein complexes bind enhancers present in the promoters of early response genes such as the high-affinity Fcγ receptor gene (FcγRI). Treatment of human peripheral blood monocytes or basophils with interleukin-3 (IL-3), IL-5, IL-10, or granulocyte-macrophage colony-stimulating factor (GM-CSF) activated DNA binding proteins that recognized the IFN-γ response region (GRR) located in the promoter of the FcγRI gene. Although tyrosine phosphorylation was required for the assembly of each of these GRR binding complexes, only those formed as a result of treatment with IFN-γ or IL-10 contained p91. Instead, complexes activated by IL-3 or GM-CSF contained a tyrosine-phosphorylated protein of 80 kilodaltons. Induction of FcγRI RNA occurred only with IFN-γ and IL-10, whereas pretreatment of cells with GM-CSF or IL-3 inhibited IFN-γ induction of FcγRI RNA. Thus, several cytokines other than interferons can activate putative transcription factors by tyrosine phosphorylation.

Nuclear or whole-cell extracts prepared from human monocytes incubated with either IFN-γ or IFN-α contain a protein or proteins (FcRFγ) that specifically recognize the GRR in the promoter of the high-affinity immunoglobulin G Fc receptor gene (*1–3*). Within the FcRFγ complex is a 91-kD tyrosine-phosphorylated protein that is a component of the ISGF-3 transcription complex, which causes IFN-α–stimulated expression of early response genes (*2–4*). Because the peripheral blood monocyte is a critical target cell for IFN-α, IFN-γ, and other cytokines, experiments were done to determine whether any cytokines other than the interferons might induce the formation of FcRFγ. Whole-cell extracts were prepared from monocytes incubated with various cytokines for 15 min at 37°C and analyzed by electrophoretic mobility–shift assays (EMSAs) with a ^{32}P-labeled oligonucleotide corresponding to the GRR (Fig. 1A) (*5*). Untreated cells showed no formation of FcRFγ, whereas extracts prepared from monocytes treated with IL-3 or GM-CSF contained GRR binding complexes that migrated with a mobility different than that of the FcRFγ (Fig. 1A) complex observed after IFN-γ activation. In contrast, IL-10 activated the formation of a GRR binding complex with a mobility similar to that of FcRFγ. Other cytokines that have effects on monocytes—IL-1, IL-2, IL-6, tumor necrosis factor (TNF), monocyte colony-stimulating factor (M-CSF), and lipopolysaccharide—showed no formation of GRR binding complexes.

Binding of FcRFγ and the complexes activated by GM-CSF treatment of monocytes was inhibited by addition of excess unlabeled GRR (Fig. 1B), but not by addition of an unlabeled oligonucleotide corresponding to the IFN-γ activation sequence (GAS) within the promoter of the guanylate-binding protein gene (Fig. 1B) (*6*). The complexes induced by treatment of monocytes with IL-3 and IL-10 showed similar binding specificities (*7*). When the GAS oligonucleotide was used as a probe, only

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