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TREATMENT OF RHEUMATOID ARTHRITIS WITH A RECOMBINANT HUMAN TUMOR NECROSIS FACTOR RECEPTOR (p75)-Fc FUSION PROTEIN

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ABSTRACT

Background Tumor necrosis factor (TNF) is a proinflammatory cytokine involved in the pathogenesis of rheumatoid arthritis, and antagonism of TNF may reduce the activity of the disease. This study evaluated the safety and efficacy of a novel TNF antagonist — a recombinant fusion protein that consists of the soluble TNF receptor (p75) linked to the Fc portion of human IgG1 (TNFR:Fc).

Methods In this multicenter, double-blind trial, we randomly assigned 180 patients with refractory rheumatoid arthritis to receive subcutaneous injections of placebo or one of three doses of TNFR:Fc (0.25, 2, or 16 mg per square meter of body-surface area) twice weekly for three months. The clinical response was measured by changes in composite symptoms of arthritis defined according to American College of Rheumatology criteria.

Results Treatment with TNFR:Fc led to significant reductions in disease activity, and the therapeutic effects of TNFR:Fc were dose-related. At three months, 75 percent of the patients in the group assigned to 16 mg of TNFR:Fc per square meter had improvement of 20 percent or more in symptoms, as compared with 14 percent in the placebo group ($P < 0.001$). In the group assigned to 16 mg per square meter, the mean percent reduction in the number of tender or swollen joints at three months was 61 percent, as compared with 25 percent in the placebo group ($P < 0.001$). The most common adverse events were mild injection-site reactions and mild upper respiratory tract symptoms. There were no dose-limiting toxic effects, and no antibodies to TNFR:Fc were detected in serum samples.

Conclusions In this three-month trial TNFR:Fc was safe, well tolerated, and associated with improvement in the inflammatory symptoms of rheumatoid arthritis. (N Engl J Med 1997;337:141-7.)

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RHEUMATOID arthritis is a common disease, and it produces substantial morbidity as well as an increase in mortality.¹⁻⁴ Although the causes of rheumatoid arthritis are not fully understood, laboratory and clinical evidence suggests that proinflammatory cytokines, particularly tumor necrosis factor (TNF), have an important role in its pathogenesis.^{5,6} TNF induces the release of matrix metalloproteases from neutrophils, fibroblasts, and chondrocytes⁷⁻⁹; induces the expression of endothelial adhesion molecules involved in the migration of leukocytes to extravascular sites of inflammation¹⁰; and stimulates the release of other proinflammatory cytokines.^{11,12} TNF concentrations are increased in the synovial fluid of persons with active rheumatoid arthritis,^{13,14} and increased plasma levels of TNF are associated with joint pain.¹⁵ Administration of TNF antagonists to patients with rheumatoid arthritis has been shown to reduce symptoms.¹⁶⁻¹⁹

There are two distinct cell-surface TNF receptors (TNFRs), designated p55 and p75.^{20,21} Soluble, truncated versions of membrane TNFRs, consisting of only the extracellular, ligand-binding domain, are present in body fluids and are thought to be involved in regulating TNF activity.^{22,23} Soluble TNFRs have been detected in synovial tissue and at the junction between cartilage and pannus.^{24,25} Their levels are increased in serum and synovial fluid in rheumatoid arthritis²⁶⁻²⁹ and in many other autoimmune and inflammatory conditions.³⁰⁻⁴⁴

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A recombinant human TNFR p75-Fc fusion protein (TNFR:Fc) (Enbrel, Immunex, Seattle) has been developed for therapeutic neutralization of TNF.⁴⁵ DNA encoding the soluble portion of human TNFR p75 was linked to DNA encoding the Fc portion of a human IgG1 molecule, and the combined DNA was then expressed in a mammalian cell line. The resulting immunoglobulin-like dimer, composed exclusively of human amino acid sequences, acts as a competitive inhibitor of TNF and prevents binding of TNF to the cell-surface TNFR, thereby reducing the biologic activity of TNF. Safety studies in normal human volunteers revealed no adverse effects after the intravenous administration of TNFR:Fc.⁴⁶ There were trends toward a reduction in disease activity in a safety and dose-finding study of TNFR:Fc administered for four weeks to a small number of patients with refractory rheumatoid arthritis.⁴⁷ On the basis of these findings, we undertook a multicenter, randomized, double-blind, placebo-controlled trial of TNFR:Fc in patients with active, refractory rheumatoid arthritis.

METHODS

Patients

Men and women 18 years of age or older were eligible if they met the criteria of the American Rheumatism Association for rheumatoid arthritis⁴⁸ and were in functional class I, II, or III according to the criteria of the American College of Rheumatology.⁴⁹ All candidates had been unsuccessfully treated (lack of efficacy) with between one and four of the following disease-modifying antirheumatic drugs: hydroxychloroquine, oral or injectable gold, methotrexate, azathioprine, penicillamine, and sulfasalazine. No such therapy was allowed during a four-week washout period before the first dose of study drug. Patients receiving nonsteroidal antiinflammatory drugs (NSAIDs), corticosteroids (≤ 10 mg per day), or both were eligible if the dosage had been stable for at least four weeks before day 1 of the washout period and remained so throughout the study and follow-up period. Requisite base-line laboratory values included a hemoglobin level of at least 8.5 per liter, platelet count of at least 125,000 per cubic millimeter, white-cell count of at least 3500 per cubic millimeter, a serum creatinine level of not more than 2 mg per deciliter (177 μmol per liter), and liver aminotransferase levels not more than twice the laboratory's upper limit of normal. The necessary degree of disease activity at enrollment (before washout) was confirmed by a finding of 10 or more swollen joints, 12 or more tender joints, and one of the following two criteria: a Westergren erythrocyte sedimentation rate of at least 28 mm per hour or a serum C-reactive protein level of more than 2.0 mg per deciliter; or morning stiffness for at least 45 minutes. Sexually active men and sexually active premenopausal women, except for men or women who had undergone surgical sterilization, were required to use a medically accepted form of contraception by the time of enrollment and to continue its use through follow-up. Women in this category also had to have a negative serum pregnancy test within five days before the first dose of study drug.

Study Protocol

The study protocol was approved by the human-research committee at each participating center. Before the start of the washout period, patients gave written informed consent, had a complete medical history taken, and underwent a complete physical examination. A hematology profile (complete blood count, differential

count, and platelet count), serum chemical profile (blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, total protein, and albumin), and analysis of a clean-catch urine specimen with microscopical analysis were also completed. Base-line clinical assessments included the following: complete count of swollen and tender joints (71 joints evaluated; cervical spine and hips evaluated only for tenderness); duration of morning stiffness; health-assessment questionnaire⁵⁰; physician's and patient's global assessment, on a scale from 0 (asymptomatic) to 10 (severe symptoms); patient's assessment of pain, on a visual-analogue scale from 0 (no pain) to 10 (severe pain)⁵⁰; Westergren erythrocyte sedimentation rate; and C-reactive protein level.⁵¹ Disease-activity assessments were repeated on day 1 of treatment and every two weeks or monthly throughout the study. Assessments were performed by rheumatologists or trained nurse coordinators. To minimize variation between observers, each patient's disease activity was assessed primarily by the same person throughout the study.

A patient could be withdrawn from the trial at any time after enrollment for the following reasons: the patient's request, pregnancy, serious infection, severe or life-threatening adverse event, or inadequate control of arthritis symptoms (≥ 50 percent increase in the total number of swollen or tender joints) necessitating an increase in the systemic corticosteroid dosage or reinstitution of therapy with disease-modifying antirheumatic drugs.

Follow-up evaluations after the discontinuation of therapy at three months were completed every two weeks for one month, then once a month until the patient required new or previous antirheumatic therapy or until the total count of swollen and tender joints returned to the base-line value. Hematologic testing, serum chemistry, urinalysis, and anti-TNFR:Fc antibody testing were repeated periodically during the trial, including follow-up, and on the day the patient required initiation or reinstitution of therapy with disease-modifying antirheumatic drugs or the joint count returned to the base-line value. All patients were evaluated for side effects and laboratory abnormalities.

Treatment

Patients were randomly assigned to one of four treatment groups: placebo; 0.25 mg of TNFR:Fc per square meter of body-surface area (the 0.25-mg group); 2 mg of TNFR:Fc per square meter (the 2-mg group); or 16 mg of TNFR:Fc per square meter (the 16-mg group). The study drug or placebo was injected subcutaneously twice weekly for three months. TNFR:Fc was supplied as a sterile lyophilized powder containing 10 mg of TNFR:Fc, 40 mg of mannitol, 10 mg of sucrose, and 1.2 mg of TRIS (tromethamine) per vial. The placebo was a lyophilized powder containing 40 mg of mannitol, 10 mg of sucrose, and 1.2 mg of TRIS. The injection volume was standardized for all patients by dilution with bacteriostatic water for injection (two 1.5-ml injections per dose). Injections were given at approximately the same time in the morning on a Monday-Thursday or Tuesday-Friday schedule.

Concomitant Medications

In addition to stable doses of NSAIDs and corticosteroids, the following analgesic medications were allowed throughout the trial, except on the day before a joint evaluation: acetaminophen with codeine phosphate, acetaminophen with propoxyphene napsylate, and acetaminophen with oxycodone hydrochloride.

Antibody Testing

Serum samples were collected for antibody testing on day 1 (before treatment), at three months (end of treatment), and two to four weeks after treatment ended.

Wells of polystyrene microtiter plates (Maxisorp, Nunc, Roskilde, Denmark) were coated with 63 ng of TNFR:Fc per milliliter in 0.01 M phosphate-buffered saline, pH 7.2, and incubated overnight at 2 to 8°C. The plates were washed with phosphate-buffered saline, and controls and samples diluted in phosphate-buffered saline with 5 percent normal goat serum were added and incubated

in the wells for one hour at room temperature. After a second washing with phosphate-buffered saline, a peroxidase-labeled F(ab')₂-specific goat antihuman immunoglobulin conjugate (Jackson ImmunoResearch Laboratories, West Grove, Pa.) was added, and the plates were incubated for another hour at room temperature. After a final washing with phosphate-buffered saline, *o*-phenylenediamine dihydrochloride peroxidase substrate–chromogen solution was added to the wells for a 10-minute incubation at room temperature for color development. The reaction was stopped with the addition of 1 M phosphoric acid, and optical densities were determined at 490 nm. Test samples were run in duplicate at dilutions of 1:50, 1:100, 1:200, and 1:400. A rabbit anti-TNFR polyclonal serum, along with a heterologous antirabbit immunoglobulin detection reagent, was used as a positive control for detection of TNFR:Fc coated onto the plates. Wells coated with human IgG served as controls for the conjugate. Samples were scored as positive if two or more of the dilutions of the post-treatment sample or samples had optical-density values at least four times as high as those of the corresponding pretreatment-sample dilutions. The ability to detect antibodies to TNFR:Fc was confirmed by the presence of antibodies in serum samples obtained from monkeys treated with TNFR:Fc and tested with the same reagents as those used for the detection of human antibodies.

Development of autoantibodies has been reported with the administration of other inhibitors of TNF.⁵² Tests for autoantibodies were not performed.

Statistical Analysis

The percent change from base line to three months (day 85) in the swollen-joint count, tender-joint count, and total count of swollen or tender joints was the primary measure of efficacy. Secondary end points included pain, quality of life, duration of morning stiffness, erythrocyte sedimentation rate, C-reactive protein level, and physician's and patient's global assessments. If a subject withdrew from the study, the last available value was used as the three-month value. The data were also analyzed to determine the number of patients meeting American College of Rheumatology criteria for 20 and 50 percent improvement, which specify 20 and 50 percent reductions in the number of swollen or tender joints and the same degree of improvement in at least three of five other variables: pain, degree of disability according to the health-assessment questionnaire, patient's global assessment, physician's global assessment, and erythrocyte sedimentation rate or C-reactive protein level.⁵¹ For these end points, subjects who dropped out were considered to have had no response.

For changes from base line, the four treatment groups were compared by analysis of variance, with adjustment for treatment and study site. Treatment differences were consistent across study sites. Response rates for American College of Rheumatology criteria were compared by the chi-square test. Six pairwise comparisons of efficacy were made for each end point. With the Bonferroni correction for multiple comparisons, the P value had to be less than 0.008 for significance at the 0.05 level to be retained.

The sample size of 180 subjects (45 in the placebo group and 135 in the three TNFR:Fc groups) was chosen on the basis of the variability in the percent change from base line in the total count of swollen or tender joints determined in a previous trial.⁴⁷ Assuming a standard deviation of 40 for the percent change in the total count and assuming a difference in this change between the placebo and treatment groups of 20 percentage points (that is, a mean reduction of 20 percent in the placebo group and 40 percent in the treated patients), the sample size was sufficient to detect such differences between treatment (n = 135) and placebo (n = 45) groups with 82 percent power.

RESULTS

Characteristics of the Patients

The characteristics of the patients before treatment are summarized in Table 1. Forty-eight men

and 132 women were enrolled in the trial. Their mean age was 53 years, and 77 percent had disease of more than 5 years' duration. No significant differences between groups were detected in pretreatment characteristics or base-line disease activity. Seventy-six percent of the patients completed TNFR:Fc treatment (61 percent in the 0.25-mg group, 78 percent in the 2-mg group, and 93 percent in the 16-mg group), as compared with 52 percent of the patients assigned to placebo. The primary reason for withdrawal was inadequate control of arthritis symptoms. Among the patients receiving TNFR:Fc, the proportions of patients who withdrew because of inadequate symptom control were 35 percent in the 0.25-mg group, 17 percent in the 2-mg group, and 5 percent in the 16-mg group; among the patients receiving placebo, it was 43 percent.

Efficacy

TNFR:Fc produced significant improvement in all measures of disease activity (Table 2). A clear dose–response relation was observed in the numbers of swollen or tender joints, and patients who received the highest dose of TNFR:Fc had the greatest improvement. In the 16-mg group, the mean percent reduction in the total count at three months was 61 percent, as compared with 25 percent in the placebo group (P < 0.001). Figures 1 and 2 depict the numbers of swollen and tender joints as a function of time in each treatment group. In the placebo and 0.25-mg groups, there was an initial response, but no improvement was noted thereafter. The 16-mg dose of TNFR:Fc was associated with the greatest reduction in the number of swollen or tender joints. This difference was apparent by the end of week 2 and was most pronounced at the end of treatment (at three months).

TNFR:Fc treatment was also associated with significant reductions in pain and duration of morning stiffness, significant improvement in the quality of life and physician's and patient's global assessments, and significant reductions in disease activity as assessed by objective laboratory measures (erythrocyte sedimentation rate and C-reactive protein level) (Table 2). According to American College of Rheumatology criteria, at three months 57 percent of the 16-mg group had at least 50 percent improvement, as compared with 7 percent of the placebo group (P < 0.001); 75 percent of the 16-mg group had at least 20 percent improvement, as compared with 14 percent of the placebo group (P < 0.001) (Table 3). Measures of disease activity moved toward base-line levels after the cessation of TNFR:Fc therapy.

Safety and Tolerability

TNFR:Fc was well tolerated; no dose-limiting toxic effects were observed. Only one patient withdrew because of an adverse event related to TNFR:Fc

TABLE 1. DEMOGRAPHIC AND BASE-LINE CLINICAL CHARACTERISTICS OF THE STUDY PATIENTS.*

CHARACTERISTIC	PLACEBO	TNFR:Fc		
		0.25 mg/m ²	2 mg/m ²	16 mg/m ²
No. of patients	44	46	46	44
Mean age (yr)	55	54	52	52
Female sex (%)	82	70	61	82
White race (%)	91	96	100	93
Duration of disease (%)				
<2 yr	11	7	4	4
2–5 yr	18	17	15	16
>5 yr	71	76	80	80
Measures of arthritis activity				
Swollen-joint count (no.)	22±9	24±11	24±9	24±11
Tender-joint count (no.)	29±13	32±14	32±13	30±15
Total count (no.)	51±19	56±23	55±21	54±25
Morning stiffness (hr)	4.9	4.3	5.2	4.9
Physician's assessment†	7.0	7.4	7.2	6.5
Patient's assessment†	6.7	7.1	6.9	6.5
Pain (visual analogue)‡	6.4	6.9	6.7	6.3
Quality of life (HAQ)‡	146	153	138	135
Erythrocyte sedimentation rate (mm/hr)	40	44	36	35
C-reactive protein (mg/dl)	3.9	4.1	3.6	3.6
Medications in year before study (%)				
Corticosteroids	75	76	80	91
DMARDs	100	100	100	100
Methotrexate	34	41	30	27
NSAIDs	75	80	70	75
Concurrent medications (%)				
Corticosteroids	66	59	65	77
NSAIDs	73	70	80	75
Acetaminophen with codeine phosphate	25	33	33	18
Acetaminophen with propoxyphene napsylate	34	39	30	30
Acetaminophen with oxycodone hydrochloride	11	24	26	7

*HAQ denotes health-assessment questionnaire, DMARDs disease-modifying antirheumatic drugs, and NSAIDs nonsteroidal antiinflammatory drugs. Plus-minus values are means ±SD.

†On this scale, 0 is best and 10 worst.

‡On this scale, 45 is best and 245 worst.

(a mild injection-site reaction). Adverse events related or potentially related to TNFR:Fc were limited to mild injection-site reactions (erythema or erythema plus discomfort) and mild upper respiratory tract symptoms (cough, rhinitis, sinusitis, upper respiratory tract infection, and pharyngitis). Injection-site reactions generally occurred with only one of the several injections given in the three-month treatment period and resolved in two to three days. Upper respiratory tract symptoms were transient, resolved without interruption of TNFR:Fc dosing, and occurred most frequently in the 2-mg and 16-mg groups; other, nonrespiratory infections were rare and never serious. One patient died during the study, a 72-year-old patient receiving placebo.

No major abnormalities in hematologic findings or serum chemical profiles were noted during or after the study; in fact, dose-related improvements in anemia and dose-related decreases in platelet counts were seen, which reflect a reduction in disease activity.

No antibodies to TNFR:Fc were detected in serum samples from any patient tested.

DISCUSSION

The results of this randomized, double-blind trial show the clinical efficacy of a soluble recombinant human TNFR p75-Fc fusion protein in patients with active rheumatoid arthritis. Treatment with TNFR:Fc for three months was associated, in a dose-related fashion, with a reduction in disease activity as assessed by a number of clinical end points, biochemical markers of disease, and quality of life. Taken together with results obtained in preclinical models⁵³ and trials with monoclonal antibodies to TNF,¹⁶⁻¹⁹ these data show that TNF antagonism is a valid approach to the treatment of rheumatoid arthritis. In addition, these data demonstrate the usefulness of a soluble cytokine receptor in human disease.

The mechanism of action of TNFR:Fc in rheumatoid arthritis probably involves its ability to inhibit competitively TNF binding to cell-surface TNFR. On the basis of results of preclinical and clinical studies in which TNF concentrations were determined in biologic fluids after the administration of TNFR:Fc, it is clear that TNFR:Fc does not pro-

TABLE 2. EFFECT OF TREATMENT ON MEASURES OF DISEASE ACTIVITY AT THREE MONTHS.

MEASURE OF ACTIVITY*	PLACEBO	TNFR:Fc			P VALUE†
		0.25 mg/m ²	2 mg/m ²	16 mg/m ²	
No. of patients	44	46	46	44	
Swollen-joint count (no.)	17	19	17	11	<0.001
Tender-joint count (no.)	22	24	17	13	<0.001
Total count (no.)	39	43	34	24	<0.001
Morning stiffness (hr)	4.1	5.3	2.6	1.1	0.004
Physician's assessment‡	5.9	5.6	4.3	2.7	<0.001
Patient's assessment‡	6.2	5.8	4.6	3.2	<0.001
Pain (VAS)‡	6.1	5.6	4.6	3.1	0.001
Quality of life (HAQ)§	141	137	123	104	<0.001
Erythrocyte sedimentation rate (mm/hr)	40	39	27	21	<0.001
C-reactive protein (mg/dl)	2.6	2.4	2.0	0.9	<0.001
Improvement from base line (%)¶					
Swollen-joint count	24	16	32	58	<0.001
Tender-joint count	28	25	46	64	<0.001
Total count	25	22	40	61	<0.001

*VAS denotes visual-analogue scale, and HAQ health-assessment questionnaire.

†Data reflect an intention-to-treat analysis. The P values were obtained from an analysis of variance comparing all four treatment groups in terms of the percent change from base line to three months with a model that contained main effects of treatment and center. For each patient, missing values were replaced by the last available value. In the analyses of erythrocyte sedimentation rate and C-reactive protein level, only patients with observed values were included.

‡On this scale, 0 is best and 10 worst.

§On this scale, 45 is best and 245 worst.

¶Percent improvement from base line was calculated as the average of the changes in the individual patients, not as the difference between the group means at base line and month 3.

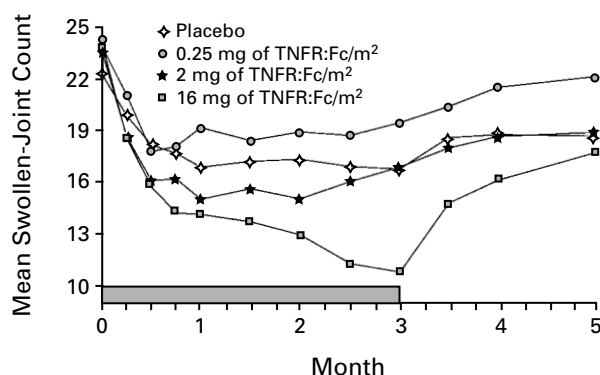


Figure 1. Mean Swollen-Joint Count.

The shaded bar represents the treatment period. For each patient, missing values were replaced by the last available value.

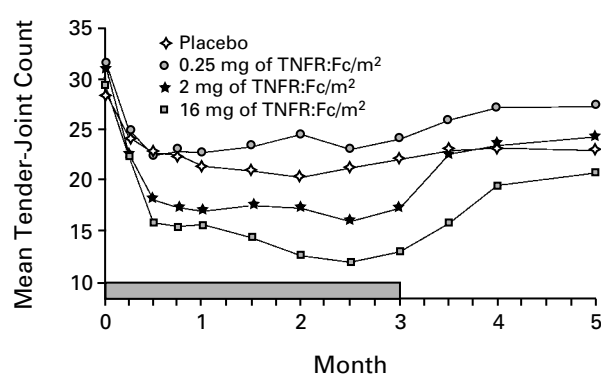


Figure 2. Mean Tender-Joint Count.

The shaded bar represents the treatment period. For each patient, missing values were replaced by the last available value.

mote rapid removal of TNF.^{45,54} In animals and in humans, the administration of TNFR:Fc has been shown to prolong the half-life of TNF; however, it also renders the TNF biologically unavailable.^{45,54} Thus, TNFR:Fc acts as both a cytokine “carrier” and a TNF antagonist. Another characteristic of TNFR:Fc that might influence its activity is the ability to bind

to Fc receptors. However, the potential for Fc-receptor binding of TNFR:Fc in vivo is probably minimal because of the high concentration of immunoglobulin in human plasma. Another potentially important consideration is the capacity of TNFR:Fc to bind to another inflammatory cytokine in the TNF family, lymphotoxin- α (TNF- β).⁵⁵ It is conceivable

TABLE 3. PROPORTIONS OF PATIENTS WITH 20 AND 50 PERCENT IMPROVEMENT ACCORDING TO AMERICAN COLLEGE OF RHEUMATOLOGY CRITERIA.

AMOUNT OF IMPROVEMENT	PLACEBO	TNFR:Fc			P VALUE*
		0.25 mg/m ²	2 mg/m ²	16 mg/m ²	
No. of patients	44	46	46	44	
	percent of patients				
20%					
1 mo	20	28	48	59	0.001
2 mo	16	28	43	68	<0.001
3 mo	14	33	46	75	<0.001
50%					
1 mo	2	9	15	25	0.011
2 mo	5	9	24	27	0.006
3 mo	7	9	22	57	<0.001

*The P values were obtained by chi-square test comparing all four treatment groups.

that antagonism of lymphotoxin- α contributes to the effect of TNFR:Fc.

Soluble cytokine receptors may have a distinct advantage over other TNF-neutralizing agents, such as monoclonal antibodies, in terms of immunogenicity. A major concern in monoclonal-antibody therapy is the potential for patients to form antibodies that neutralize the therapeutic agent, limiting its long-term usefulness or causing allergic reactions on re-treatment. However, the use of soluble cytokine receptors or receptor-Fc fusion constructs containing only human amino acid sequences may obviate this concern. Indeed, no antibodies against TNFR:Fc were detected in previous studies involving patients with rheumatoid arthritis and normal volunteers, nor were any found in the present study, after three months of treatment and throughout the follow-up period. The ability of the assay procedure to detect such antibodies is confirmed by the relative ease with which heterologous, nonhuman-primate antibodies to TNFR:Fc were detected in toxicology studies (unpublished data).

TNFR:Fc produced significant, rapid, and sustained reductions in disease activity. A strong, consistent dose-response relation was seen in most variables measured. The few adverse events noted — injection-site reactions and upper respiratory tract symptoms — were mild and easily managed. Cessation of therapy was associated with an increase in disease activity, suggesting that continued administration of TNFR:Fc is necessary for sustained effect. This hypothesis is consistent with recent data showing loss of effect of a monoclonal anti-TNF antibody, which disappeared from the circulation over a period of several weeks after a single high-dose intravenous bolus injection.^{16,56} In the case of TNFR:Fc, the relatively rapid loss of effect after drug withdrawal may prove to be

a desirable characteristic for a TNF antagonist in future regimens involving long-term administration, should TNF antagonism prove to be associated with a clinically undesirable side effect.

Given the compelling evidence that proinflammatory cytokines are involved in the pathogenesis of rheumatoid arthritis, it is reasonable to propose that interference with the cytokine cascade earlier in the course of the disease may be of additional therapeutic benefit. Further investigation is therefore warranted to determine whether earlier, long-term treatment with TNFR:Fc can prevent or delay the debilitating consequences of this disease.

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