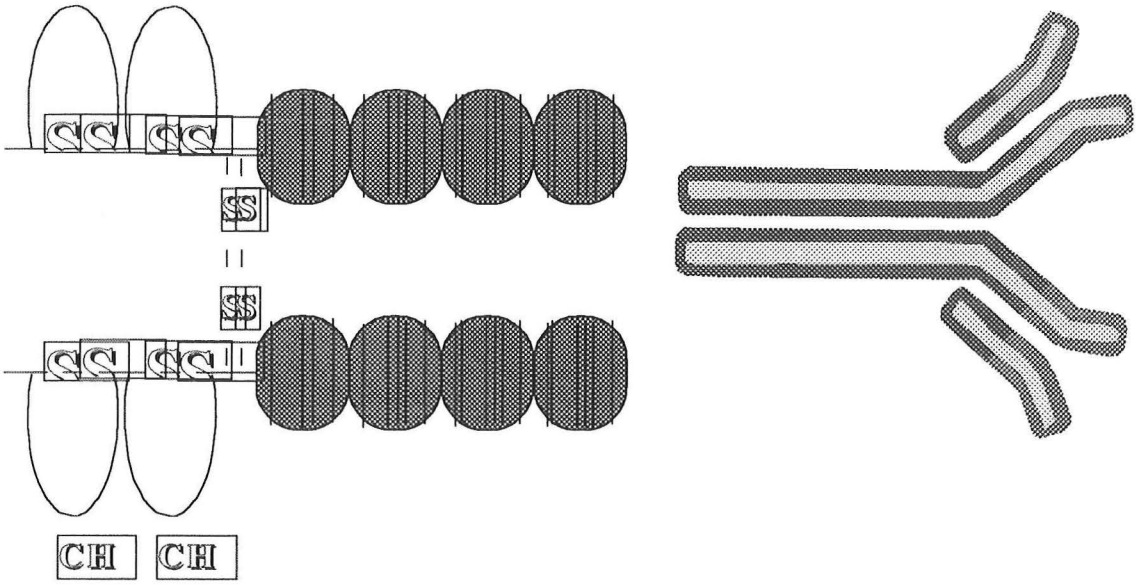
New Methods to Treat Inflammation in the Rheumatic Diseases



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Research Interests:
Regulation of inflammation by transcription factors.
Regulation of B lymphocyte differentiation.

Introduction

New therapies for treating inflammation have been eagerly awaited. The previously available antirheumatic agents including IM gold, penicillamine, sulfasalazine, and hydroxychloroquine have had insufficient efficacy and unacceptable toxicity in many cases. Methotrexate represented an advance in the 1980's and 1990's but even here there were significant numbers with insufficient control of joint inflammation, side effects, or ongoing bone damage. The newest approach is now the targeting of specific inflammatory cytokines for neutralization with antibodies, soluble receptors, or receptor antagonists. These agents are providing relief of inflammation to patients with rheumatoid arthritis and, increasingly, to patients with multiple other forms of arthritis or even nonrheumatic inflammatory conditions.

Rheumatoid arthritis

Rheumatoid arthritis is the most common systemic rheumatic disease, affecting about 1% of the population or more than 2 million Americans. It affects females 3 times more often than males and has a peak onset at the ages of 35 to 45 years. Since the causative agent is unknown, RA is diagnosed clinically based on four of these criteria being present for at least 6 weeks:

1. Morning stiffness of at least 1 hour
2. Arthritis of 3 or more joint areas
3. Arthritis of the PIP, MCP, or wrist joints
4. Symmetric arthritis
5. Subcutaneous nodules
6. Positive rheumatoid factor
7. Radiographic erosions or periarticular osteopenia in hands or wrists.

A poor prognosis for the course of RA is found in those with severe disease. Specific factors predicting a poor outcome include a generalized polyarthritis in 10 to 20 total joints, extra-articular disease such as nodules and vasculitis, persistent elevation of inflammatory markers such as the ESR and CRP, rheumatoid factor positivity, erosions within 2 years of disease onset, HLA-DR4 genotype, HAQ (health assessment questionnaire) score > 1, and education below 11^{th} grade level. RA shortens survival and frequently leads to disability. The lifespan of RA patients is shortened by 3 to 18 years. In the 1980's it was pointed out that the life expectancy of RA patients is comparable to those diagnosed with stage IV Hodgkins disease before the use of chemotherapy or three vessel coronary disease before the widespread use of surgical revascularization [1]. The most common cause of mortality in RA is cardiovascular disease, at a frequency similar to that of the rest of the population. However, there is a five-fold increase in infections, and a 5 to 8 fold increase in malignancies. Other causes of mortality seen at higher levels are amyloidosis, GI bleeding from NSAIDs, and complications of RA. Disability (functional class III or IV) is seen in 50% of RA patients within 10 years and in up to 90% with long-term disease. One-third of working patients give up their jobs within 5 years, leading to a mean 15% cumulative loss in earnings potential in this young

population. Aggressive DMARD therapy in the age before TNFα blockade reduced disability by 30%.

Pathogenesis

No etiologic agent for RA has been found. The focus in treating the condition therefore falls on control of inflammation itself. Rheumatoid synovium contains a large number of cytokines, with IL-4 the only notably absent one. Proinflammatory cytokines include TNFα, IL-1, IL-6; anti-inflammatory mediators include IL-10, TGFβ, and IL-1ra; chemokines include IL-8, MIP-1α, MCP-1, and RANTES; and growth factors such as VEGF, PDGF, and FGF are present (Figure 1).

To determine the primacy of particular cytokines in this mixture, RA synovial samples were cultured in vitro to measure spontaneous production of cytokines. This analysis yielded high levels of TNFα, IL-1, IL-6, and GMCSF. Blocking the action of TNFα in this system inhibited the production of the other cytokines, placing TNFα at the top of the inflammatory pyramid. However, antagonists of IL-1 did not decrease TNFα production.

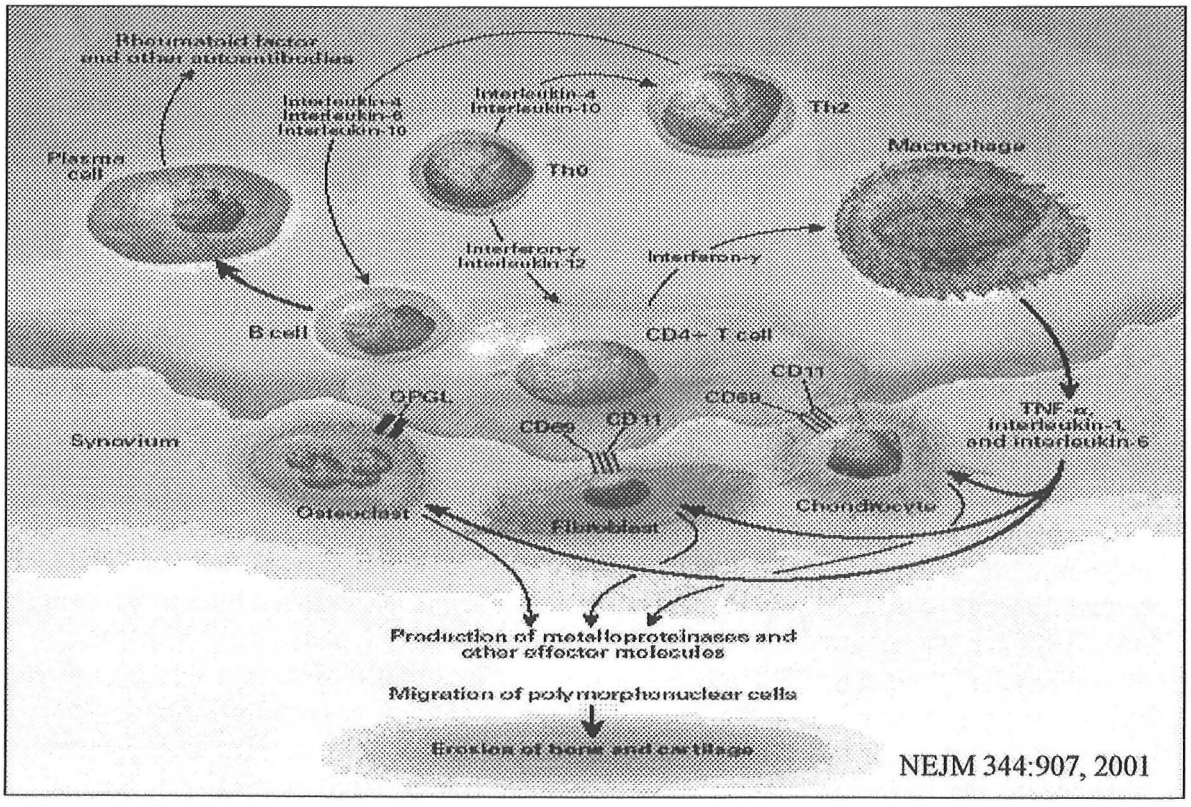

Figure 1. Pathogenesis of rheumatoid arthritis. The interaction of the immune system with chondrocytes and bone leads to joint damage.

The elaboration of TNFα by macrophages has been extensively studied in models of sepsis, in which injection of lipopolysaccharide elicits a cytokine cascade initiated by

TNFα, with subsequent production of IL-1, IL-6, and IL-8. The results from studies of RA synovium indicate significant similarities in these systems. However, despite extensive searches for infectious agents, none has been definitively identified.

A mouse model with a human TNFα transgene has been instructive to study the effects of TNFα overexpression [2]. These animals show a rheumatoid arthritis-like disease that can be suppressed by the TNFα blocker infliximab. Not only is ongoing disease controlled by this treatment, but existing bone erosions can actually be reversed. It is unknown if the human disease will respond similarly.

TNFα blockers

Two FDA-approved agents are currently available to inhibit the effects of TNFα: infliximab and etanercept. Infliximab is a mouse/human chimeric antibody molecule specific for TNFα, while etanercept is an all-human soluble TNFα receptor fused to the Fc portion of IgG1. Mechanistically, infliximab fixes complement and can lyse target cells, a feature not key to its beneficial effects in treating inflammatory arthritis but one potential reason for explaining differences in side effects between the two. Studies of the kinetics of inflammatory markers after infliximab infusion have shown that an elevated CRP can normalize within one day, that IL-1β is downregulated rapidly, and that IL-6 in the circulation drops within 4 hours. Infliximab therapy not only reduces the levels of cytokines but also diminishes inflammatory infiltrates. CD3+ T cell and macrophage numbers in synovium are reduced, and the recruitment of further inflammatory cells is inhibited by downregulation of E-selectin, IL-8, and MCP-1 expression. The altered cytokine milieu ensures that the traffic of polymorphonuclear lymphocytes to joints is greatly reduced within 2 weeks of infliximab treatment.

	Infliximab	**Etanercept**
Half-life	9.8 d	4.8 d
Binding affinity	1.8×10^9/M	10^{10}/M
Target	TNFα	TNFα/LTα
Species	Human/mouse	Human
Complement fixing	Yes	No
Lyses cells	Yes	No
Dosing	q6-8 wk IV	2x/wk sc

TNFα blocker trials in rheumatoid arthritis

Infliximab

The effectiveness of TNFα blockade in the treatment of RA has been demonstrated by several trials in the last 3 years. The ATTRACT trial studied 428 RA patients with active RA despite treatment with methotrexate (≥12.5 mg/wk) [3]. Active RA was defined as ≥6 swollen joints, ≥6 tender joints, and ≥2 of these: a.m. stiffness of at least 45 minutes, ESR ≥28, and CRP ≥2.0. The design of the trial assessed the use of

infliximab at different doses (3 or 10 mg/kg) and different dosing intervals (q 4 weeks or q 8 weeks) along with methotrexate compared with methotrexate and placebo infusions. The main outcome of the study was an ACR 20 response [4], a clinical measure of at least 20% decrease in the swollen and tender joint counts, and at least a 20% improvement in 3 or more of these 5 categories:

1. Physical disability on patient questionnaire
2. Pain score on questionnaire
3. Patient global status on questionnaire
4. Patient global status by evaluator
5. Markers of inflammation: ESR or CRP

The result of the study at 6 months was an ACR 20 response in 50 to 58% of all four groups receiving infliximab, versus a 20% response in the methotrexate plus placebo group. By 1 year of follow-up, the infliximab groups maintained a 42 to 59% ACR 20 response rate, while the MTX/placebo group was at 17% [3,5] (Figure 2). Even at 102 weeks of follow-up, the ACR 20 response in the infliximab groups remained at 40 to 48% and the MTX/placebo response was at 16%. These and further studies have demonstrated clinical effectiveness for infliximab in decreasing the signs and symptoms of the inflammatory aspects of rheumatoid arthritis.

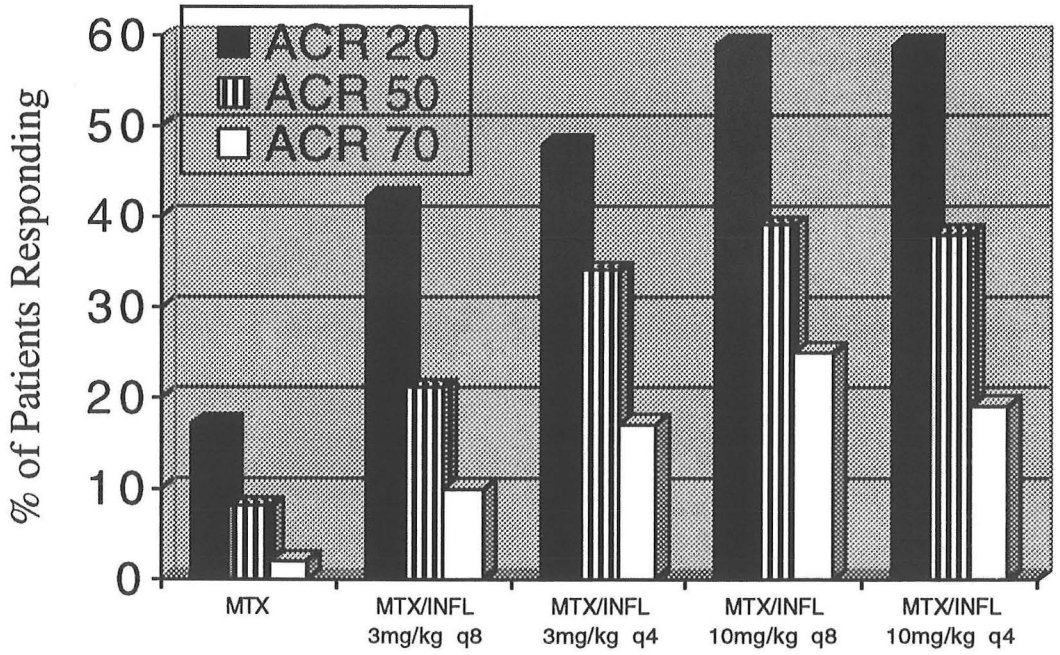

Figure 2. Clinical responses in the ATTRACT trial. ACR 20, 50, and 70 responses are shown at 54 weeks for the patient groups receiving methotrexate plus placebo (MTX), methotrexate plus infliximab at 3mg/kg every 8 weeks (MTX/INFL 3mg/kg q 8) or every 4 weeks (q4), or methotrexate plus infliximab at 10mg/kg every 8 weeks (MTX/INFL 10mg/kg q8) or every 4 weeks. All infliximab groups are statistically superior to the MTX group for all 3 levels of ACR responses.

Etanercept

Etanercept has been studied in the ERA (Etanercept in Early Erosive Rheumatoid Arthritis) Trial [6]. 632 methotrexate-naïve patients were randomized in a 2 year study to receive methotrexate (20 mg) or etanercept (10 mg or 25 mg sc 2x/week). In the third year, an open-label extension allowed all patients to receive etanercept 25 mg sc 2x/week [7]. The most complete data are available so far only for the first year of the trial. Compared to the methotrexate recipients, the patients on the higher dose of etanercept achieved a more rapid rate of improvement and were more likely to reach 20%, 50%, and 70% improvement in disease activity in the first 6 months. Between 6 months and 1 year, the differences in ACR 20, 50, or 70 responses between the 3 groups were no longer statistically significant, although the cumulative response (area under the curve for ACR-N) remained significantly greater for the higher dose etanercept groups versus the methotrexate group. The results after the open label extension in year 3 continue to show a significant improvement in ACR 20 score versus the baseline at study entry for all three treatment regimens, with no significant differences between the treatments (Figure 3). Therefore, methotrexate and etanercept are equally effective in improving joint inflammation as measured by the ACR criteria.

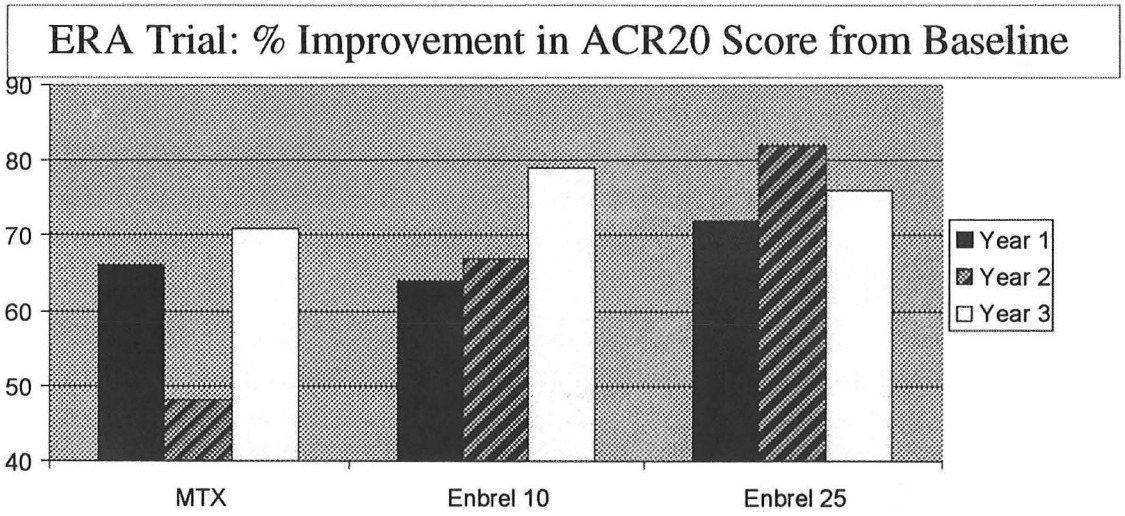

Figure 3. Clinical responses in the ERA trial. Groups of patients took methotrexate (MTX), etanercept 10 mg sc 2x/week (Enbrel 10), or etanercept 25 mg sc 2x/week (Enbrel 25). Year 3 was an open-label extension in which all patients received the higher dose etanercept.

The large trials in RA described here have also investigated the radiographic development and progression of bone erosions over the course of the studies. The ATTRACT trial demonstrated significantly less progression of joint damage in all four groups receiving infliximab plus MTX versus the group on MTX plus placebo. At 54 weeks of follow-up, there was a 9 to 10% worsening in the total radiographic score for the MTX group and no change from baseline in all four infliximab groups (Figure 4; $p < 0.001$ for all four). The beneficial effects of infliximab were seen both for components

making up the radiographic score: the presence of erosions, and joint-space narrowing. Interestingly, even patients without a clinical response to infliximab (20% decrease in number of swollen joints, the number of tender joints, or the CRP) had the full benefit of infliximab in slowing the rate of joint damage. In patients receiving methotrexate, even those with a clinical response did not show joint scores different from nonresponders. Over 102 weeks, radiographic scores continued to worsen in MTX-alone patients but were stable in the four infliximab groups.

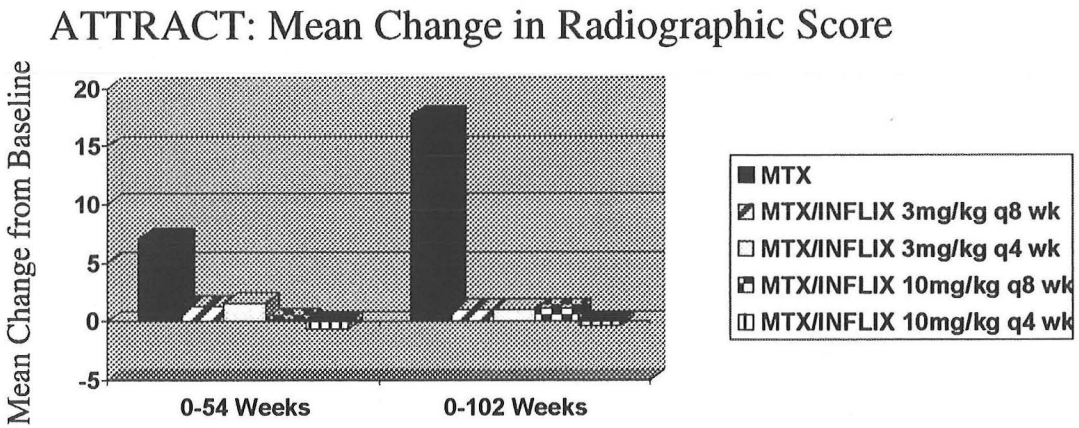

Figure 4. Radiographic progression in the ATTRACT trial. The total radiographic score continued to worsen in the methotrexate plus placebo (MTX) patients, while all four infliximab/methotrexate regimes (infused q8 weeks or q 4 weeks) significantly reduced or halted radiographic progression.

In the ERA trial, administration of etanercept led to a significantly decreased rate of bone erosions as measured by a modified Sharp's method (scoring of bone erosions in 46 joints, and joint space narrowing in 42 areas). After the first year of the ERA study, the joint erosion score had increased by 0.47 in the 25 mg etanercept group and by 1.03 in the methotrexate group ($p = 0.002$), while the joint space narrowing was similar in the two groups (Figure 5). The beneficial effect of etanercept on joint erosions was most pronounced within the first 6 months of therapy since between 6 and 12 months, methotrexate and etanercept 25 mg had equivalent effects in slowing further joint erosion. As presented in abstract form, year 2 of the ERA trial shows a continuing trend of less worsening in the total Sharp score and the erosion score in the etanercept 25 mg group vs. the methotrexate group. In the open-label third year of the trial, patients previously on methotrexate or on etanercept 10 mg were switched to etanercept 25 mg dosing. This resulted in further reductions in radiographic progression of Sharp score and erosion score.

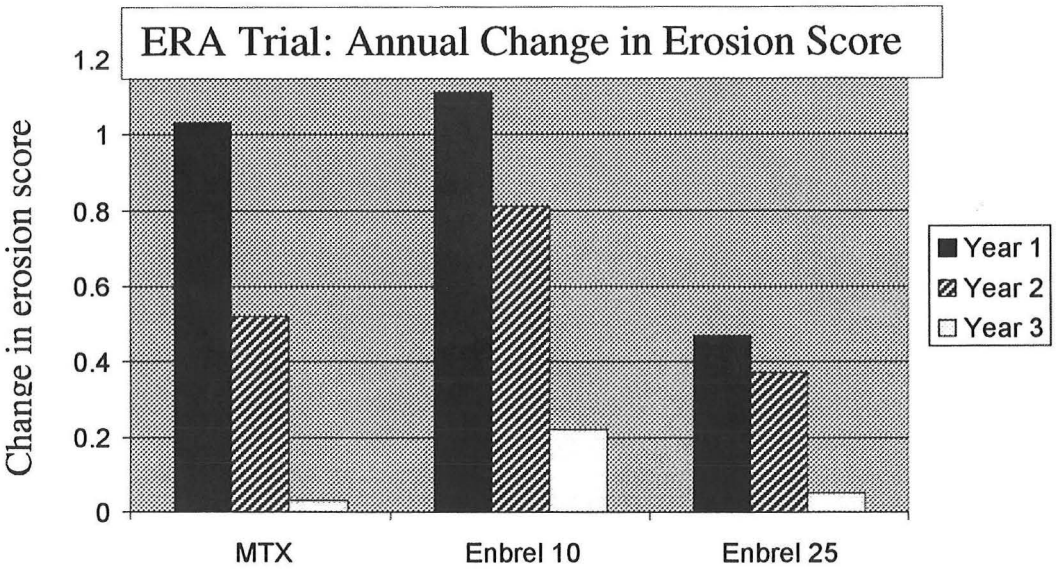

Figure 5. Radiographic progression in the ERA trial. Etanercept given 25 mg sc 2x/week (Enbrel 25) resulted in significantly less deterioration in the erosion score than did methotrexate plus placebo (MTX) or etanercept 10 mg sc 2x/week (Enbrel 10).

TNFα blockers and sepsis

The inflammatory pathway leading from the activation of TNFα and progressing to the subsequent involvement of further cytokines such as IL-1, IL-6, and IL-8 also represents the main source of pathology in sepsis and septic shock (Figure 6). In mouse models of sepsis, the injection of lipopolysaccharide leads to the activation of this same cascade of cytokines. In sufficient doses, the result is vascular leak, pulmonary edema, hypotension, and death. The primacy of TNFα in this pathway has been demonstrated by the ability of injected TNFα to recapitulate the same picture of septic shock. Nevertheless, a drawback of such a mouse model of sepsis is that whole killed bacteria or bacterial products are used to trigger the condition, while actual sepsis additionally involves control of bacterial growth.

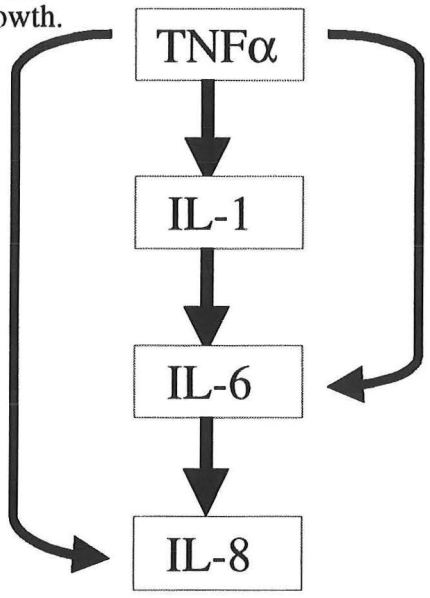

Figure 6. TNFα in inflammation. TNFα sits at the top of an inflammatory cascade, directly and indirectly inducing downstream inflammatory cytokines.

Trials in Humans

Corticosteroids

Corticosteroids inhibit TNFα production at pre- and post-transcriptional levels and therefore could have theoretical benefit in preventing excess TNFα production in sepsis. In animal models, administration of dexamethasone before challenge with LPS afforded protection by inhibiting TNFα production [8]. However, no protection was seen when the LPS was injected 15 minutes or longer after the LPS. Once the TNFα production was already well-established, the dexamethasone was not protective against TNFα toxic effects. This indicates that the timing of the corticosteroid administration is crucial in determining its efficacy.

In humans, no benefit in survival has been demonstrated in prospective, double-blind clinical trials using high-dose corticosteroids. A meta-analysis of the 9 most methodologically sound trials found that corticosteroids tend to increase mortality (RR (relative risk) 1.13, 95% confidence interval (CI) 0.99-1.29), with a trend towards increased mortality from secondary infections and for more GI bleeds [9]. This is consistent with the animal models that showed the benefits of corticosteroids in preventing the initial release of TNFα but no benefit in counteracting pre-formed TNFα. Nevertheless, subsequent studies have demonstrated some benefits from using low doses of corticosteroids, such as earlier discontinuation of vasoactive drugs in patients surviving the septic episode. No benefit to survival was described.

The theoretical benefits of low dose corticosteroids are hypothesized to stem from a relative resistance of tissues to glucocorticoids in the septic state (reviewed in [10]). Sepsis is associated with significantly elevated levels of cytokine-induced transcription factors such as NF-κB in peripheral mononuclear cells, and conversely, elevated levels of inflammatory cytokines induced by these same transcription factors. In the target organs, the transcription factors form complexes with activated glucocorticoid receptors, preventing their interaction with DNA. This resistance to glucocorticoid effects can be overcome with the addition of exogenous glucocorticoids, for example by using physiologic doses for prolonged periods.

TNFα monoclonal antibodies.

The ability to specifically block TNFα action using anti-TNFα antibodies or anti-TNFα soluble receptor provided a tool to definitively evaluate the role of TNFα in sepsis. In the NORASEPT I trial, 516 severely septic (with evidence of internal organ involvement) and 478 septic shock (hypotensive) patients received a single infusion of a murine monoclonal antibody to recombinant human TNFα (15 mg/kg or 7.5 mg/kg of BAYx1351) or placebo in a phase III randomized study [11]. When evaluated 28 days later, there was no difference in all cause mortality in the study. However, the septic shock subgroup showed a trend towards reduced mortality within the first 3 days (either dose of the mAb) that lost statistical significance when examined over the full 28 days. This trend was further investigated in the NORASEPT II trial, a randomized, double-blind,

multicenter study of 1900 patients with septic shock in which a single infusion of anti-TNF mAb (7.5 mg/kg of BAYx1351) was compared to placebo [12]. No improvement in survival was found.

A separate phase III multicenter, prospective, randomized, placebo-controlled trial using the same BAYx1351 monoclonal antibody was INTERSEPT (International Sepsis Trial Group) [13]. The results showed a mortality of 39.5% in 167 placebo patients, 31.5% in 181 patients receiving 3mg/kg anti-TNFα mAb, and 42.4% in 205 patients receiving a 15mg/kg dose (p=0.19). The differences were not statistically significant. Similarly, there was no statistical difference in mortality between treatments when septic shock patients were analyzed as a separate group. However, two prospectively-identified secondary variables did show significance in those patients who survived 28 days: shock reversal (statistically significant for both doses of anti-TNFα mAb) and time to onset of organ failure (statistically significant for the higher dose of anti-TNFα mAb only).

A fourth trial of TNFα blockers in severe sepsis or septic shock used the murine anti-TNFα monoclonal antibody fragment MAK 195F [14]. Three different doses of the antibody were compared to placebo in an open-label, randomized, dose-ranging study and again demonstrated no statistically significant effect on mortality. However, the antibody was beneficial in a dose-dependent fashion in the subgroup of patients with IL-6 levels over 1000 pg/ml. Therefore, evidence of a particularly active immune response actually led to an improved response to therapy. A similar observation has been made in patients with giant cell arteritis, who show an improved response to therapy if their initial inflammatory markers are high (higher ESR, lower Hgb and albumin) [15].

In follow-up of the importance of elevated IL-6 levels as a prognostic marker, a larger trial was designed with 2634 septic patients [16]. In results presented so far only in abstract form, 998 had IL-6 $\geq$ 1000 pg/ml and were randomized to receive the TNFα blocker afelimomab (498 patients) versus placebo (510 patients). The 28 day mortality was 43.6% in the afelimomab group, 47.6% in the placebo group, $p < 0.05$.

TNFα soluble receptors

Blockade of TNFα effects by infusion of soluble receptor constructs has been investigated in several trials of sepsis. First, the p75-human IgG1 Fc receptor fusion protein etanercept was used in a randomized study of 141 septic shock patients [17]. There was a statistically-significant, dose-dependent increase in the mortality rate of patients treated with active agent. The placebo group had a mortality of 30%, the highest p75-Fc fusion protein dose group had a mortality of 53% (p=0.014). Two other trials have utilized two different p55 TNF receptor-Fc fusion proteins. In the first, 498 with severe sepsis with or without early septic shock, or with refractory septic shock, were randomized to receive the fusion construct or placebo [18]. Using doses of TNFα receptor blockers considerably lower than in the p75-Fc fusion protein trial, there was a trend toward decreased mortality in the subgroup of patients with severe sepsis and early septic shock (reduction in mortality rate of 36%, p=0.07). A second trial in 1340 patients, using a protein with a lower TNFα neutralizing effect, did not confirm this trend [19].

Conclusions

Therefore, the use of anti-TNFα therapy in sepsis has not had a major impact on survival. Certain subsets of patients may benefit, such as those with very high levels of IL-6 and those with early rather than refractory septic shock. The choice of TNFα blocking protein may also make a difference, with current speculation centering on the importance of the protein's affinity for TNFα and the ability to lyse target cells bearing TNFα or its receptor. The ideal dose of TNFα blocker has not been established, and there are few data on whether to administer a single infusion as in all the above trials, or to dose repeatedly. Animal models predict that the most devastating consequences of sepsis can be prevented by TNFα blockade before bacterial challenge, but that later treatment is much less effective. Although TNFα sits at the peak of a pyramid of inflammatory cytokines, focusing treatment on this cytokine alone does not represent the key to combating established sepsis.

Risk of infection while using TNFα blockers

The cases of infection seen with patients on TNFα blockers point out potential limitations of this therapy and may also provide additional information about their mechanism of action.

If TNFα blockers act as general immunosuppressants, the resulting opportunistic infections should be well-known from experience with chemotherapy, HIV, or transplant patients. However, the focus in infliximab patients has been mainly on an increase in tuberculosis [20]. The role of TNFα in mouse models of tuberculosis is to regulate granuloma formation and disease containment. TNFα-deficient mice form poorly organized cellular infiltrates with extensive necrosis in response to TB, whereas wild-type mice form well-organized granulomas [21]. This mouse model provides information on an acute TB infection, whereas many of the human cases of TB in infliximab patients are presumed to be reactivation TB. It has been found that injection of TNFα antibodies into mice with "low dose" or latent TB infection leads to fatal reactivation of TB [22]. In human TB, the role of TNFα is less well-defined, although other cytokines such as IFN-γ and IL-12 are known to be protective [22-24].

As of March, 2001, infliximab had been administered to 147,000 patients, of which 121,000 (76,000 with Crohn's, 45,000 with RA) lived in the US. In this group, 70 cases of TB were reported. Although a majority of infliximab patients were receiving treatment for Crohn's disease, 47 of the TB cases were in RA patients, and 18 were in Crohn's patients. The TB was frequently not a simple pulmonary infection: it usually occurred within 3 months of beginning infliximab (Figure 7), it was extrapulmonary (especially lymph node, peritoneal, and pleural) in 56%, and disseminated in 25%. By comparison, TB in non-HIV patients is extrapulmonary in 18% and disseminated in 2%. There were 12 deaths among the 70 TB patients.

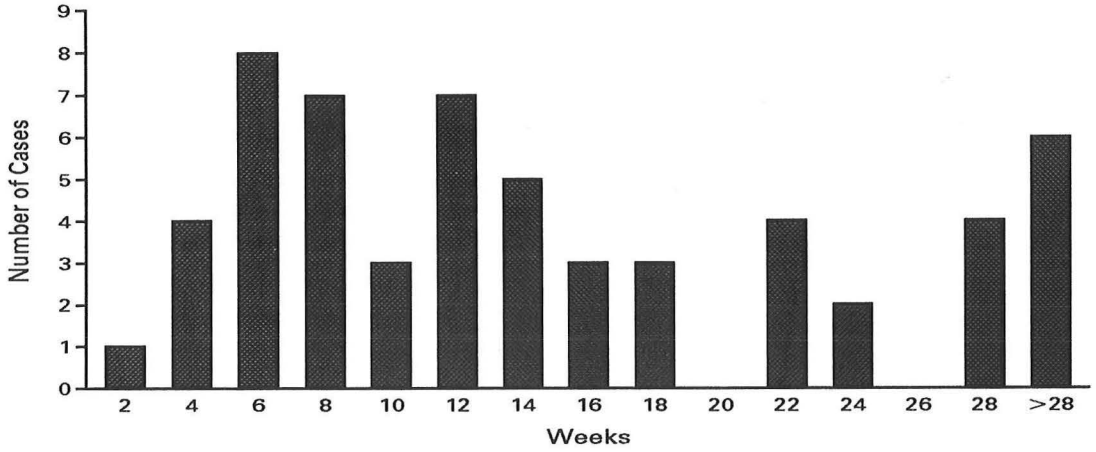

Figure 7. Time to development of TB after initiation of infliximab therapy. Most cases of TB occur within the first 14 weeks of treatment and are often presumed to be reactivation of latent TB [20].

Multiple potential explanations for the excess TB cases have been considered. First, the rate of TB in RA patients has been estimated at 6.2 cases/100,000 patients, which is indistinguishable from the 1999 overall US rate of TB [25]. However, these rates are about one fourth the rate found in the infliximab patients (24.4/100,000). Since RA patients may be treated with immunosuppressives such as prednisone, methotrexate, and azathioprine, these drugs have been considered in the pathogenesis of TB; however, the experience with RA patients on these agents has not indicated excess TB cases for the whole population of RA patients. Furthermore, other opportunistic infections beside TB were not increased in incidence. Finally, it has been observed that of the 70 TB cases, only 17 occurred in the US despite the fact that 82% of infliximab recipients were US residents. This means that most of the reported TB cases occurred outside the US. The excess TB cases cannot be ascribed to elevated TB rates in underdeveloped countries, since 64 of the 70 reported TB cases were from countries with low TB incidence. Also, immigrants were not overrepresented among the US TB cases, since only 5 of the 17 were immigrants and all had been in the US over 10 years.

The experience with etanercept has been different. Through September, 2001, there have been 13 TB (8 in US, 5 foreign) cases in 117,000 etanercept recipients worldwide [20, 26]. The 8 US cases compare with the expected incidence of 11 TB cases in this population (assuming a US incidence of TB of 6.4 cases/100,000 patient years). The differences in TB rates between etanercept and infliximab are not well-understood. Differences in dosing, route of administration, and peak serum levels may play a role. A further possibility is the different mechanisms that the two drugs have for neutralizing TNFα: etanercept is a soluble receptor and releases from its target over time, while

infliximab is an antibody that promotes complement-mediated lysis and therefore may rupture cells controlling or harboring the TB organisms.

Currently, TB screening is not routinely part of administering etanercept but has become mandatory for initiation of infliximab. Infliximab candidates should have a tuberculin skin test with a control skin test. Those with previously positive PPDs should have a CXR done. Positive PPDs must be treated before initiation of infliximab therapy.

Conclusions

Blockade of TNFα carries the theoretical risk of suppressing immune responses that normally control infections. For this reason, patients with active infections are not started on TNFα blockers and doses are held for intercurrent infections that arise during ongoing treatment. Patients taking infliximab have been shown to have an increased rate of TB. The onset of TB within the first 3 months of therapy suggests that it is often reactivation TB. Several of the TB cases have been extrapulmonary or disseminated infection, increasing the seriousness of this complication. Etanercept has not been associated with increased rates of TB, but the explanation for the difference in side effects for these two medications remains to be elucidated.

TNF blockers and spondyloarthropathy

Ankylosing Spondylitis

TNFα blockers are of theoretical usefulness in the spondyloarthropathies if TNFα represents a major inflammatory mediator in affected synovium or skin lesions. For ankylosing spondylitis (AS), both animal models and studies of human tissue samples support this view. In a transgenic mouse model overexpressing TNFα, the animals develop axial spinal disease and enthesopathy resembling human AS [27]. In studies of human AS patients, the levels of inflammatory markers such as TNFα and IL-6 are elevated in the serum compared to levels of control back pain patients [28]. Inflamed sacroiliac (SI) joint lesions contain T cells and macrophages, representing a potential source of TNFα [29]. TNFα mRNA has indeed been detected in the inflamed SI joints [30]. Elevated levels of TNFα mRNA were demonstrated in the synovium of juvenile spondyloarthropathy patients, in a comparison of spondyloarthropathy with rheumatoid arthritis, and in the sacroiliac joints of AS patients [31-33]. The balance of Th1 and Th2 responses is shifted to the Th2 arm in spondyloarthropathies, with increased production of IL-10 and decreased output of IFN-γ and IL-2. This begins to normalize with the initiation of anti-TNFα therapy. Also, spondyloarthropathies are associated with chronic inflammatory bowel disease. The inflamed gut strongly expresses TNFα, and anti-TNFα therapy is effective for the treatment of Crohn's disease [34]. Finally, there are considerable parallels with the pathogenesis of inflammation in rheumatoid arthritis, making TNFα blockade a rational therapeutic objective in ankylosing spondylitis.

Both infliximab and etanercept have been studied in the treatment of spondyloarthropathies. In uncontrolled MRI studies of spondyloarthropathy patients, etanercept treatment resulted in improvement of characteristic AS bone lesions: bone edema, entheseal lesions, and spinal edema [35]. Infliximab was studied in 70 patients in a double-blind, placebo-controlled, multicenter study of AS [36]. Patients had active disease, had been treated only with NSAIDs, and were given infliximab at 5mg/kg IV at weeks 0, 2, and 6, versus a placebo control. The observation period was 12 weeks and the primary outcome measure was a 50% improvement in the BASDAI (Bath AS disease activity index). The BASDAI measures the severity of fatigue, spinal pain, joint pain and swelling, localized tenderness, and morning stiffness; and the duration of morning stiffness. In addition, the study included multiple other measure of disease activity and inflammation: the BASFI (Bath AS functional index), a metrology index, the health-related quality of life measurement, and the CRP (C-reactive protein). The results of the study were positive. 53% of infliximab patients and 9% of placebo patients achieved the primary endpoint of 50% improvement in the BASDAI ($p < 0.001$). Patients with an elevated CRP at baseline were more likely to respond to therapy. In addition, the other measures of disease activity were significantly improved with infliximab treatment. Similar findings were reported by another group in abstract form at the 2001 American College of Rheumatology sessions [37]. Forty patients with spondyloarthropathies were enrolled in a double-blind, placebo-controlled, randomized study of infliximab 5 mg/kg at weeks 0,2, and 6 versus placebo. In this 12 week trial, infliximab was again significantly superior to placebo in: patient global assessment, physician global assessment, laboratory measures, as well as spinal and peripheral arthritis. Each of these trials had 1 case of tuberculosis.

Etanercept has been used in one randomized, placebo-controlled four month study in AS [38]. Included were 40 patients with active disease and morning stiffness of greater than 45 minutes, who did not have complete spinal fusion, and were on stable doses of medications (including DMARDs, NSAIDs, and prednisone). The primary outcome measures were a greater than 20% improvement in > 3 of these indicators: morning stiffness, nocturnal spinal pain, BASFI, patient global assessment, and swollen joint count. From the abstract of this study, it can be inferred that 80% of etanercept patients (receiving 25 mg sc b.i.w.) were responders, while about 30% of placebo patients responded ($p < 0.004$; Figure 8). Of the individual measures, there was significant improvement in morning stiffness, nocturnal spinal pain, BASFI, patient global assessment, chest expansion and occiput-wall distance, while there was no statistically significant improvement in the swollen joint score or the Schober's test. While the exact response criteria differ from the largest controlled infliximab study in AS presented above, the etanercept study did include ASAS 20, 50, and 70 responses. The ASAS20 criteria are a 20% improvement in more than three of: patient global assessment, pain assessment, BASFI, and inflammation (morning stiffness intensity and duration) without deterioration in the potential remaining domain [39]. By this measure, etanercept-treated patients achieved an ASAS 20 response in 83% (vs. 29% placebo), an ASAS 50 in 51% (vs. 16% placebo), and an ASAS 70 in 25% (vs. 12% placebo). Although not evaluated by exactly the same instruments, these two trials of etanercept and infliximab achieved a

comparable 50% response rate. The etanercept trial had no serious adverse events and no difference in side effects between study drug and placebo arms of the trial.

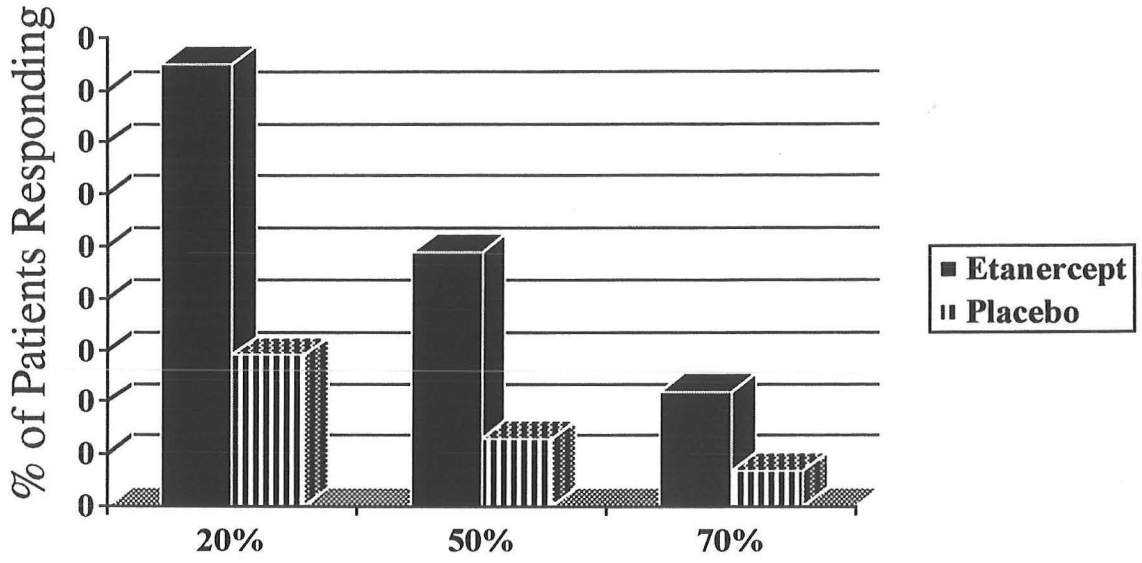

Figure 8. Use of etanercept in ankylosing spondylitis. Clinical responses were measured by the ASAS criteria as 20%, 50%, or 70% improvement [38].

Psoriasis

In the case of psoriasis, the presence of high levels of TNFα has been demonstrated in synovial tissue, in synovial fluid, and in psoriatic skin lesions [40-42] (Figure 9). Although the clinical picture of psoriatic arthritis and rheumatoid arthritis is distinguishable on the basis of a history of skin disease and the pattern of joint involvement, there are considerable similarities in the involvement of small joints, the inflammatory and destructive potential of the arthritis, and the presence of TNFα at sites of inflammation. Therefore, TNFα blockade represents a potential new therapeutic option for psoriatic arthritis, as well.

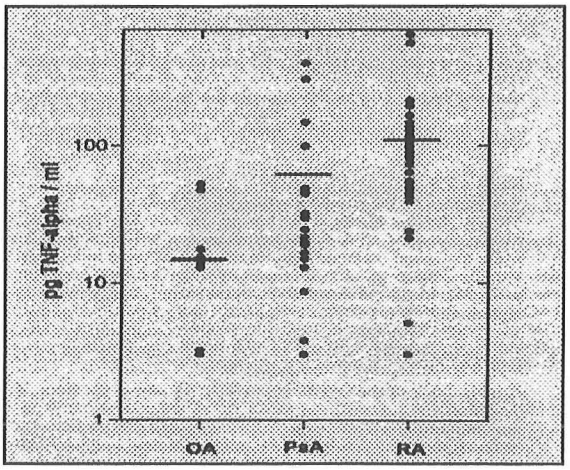

Figure 9. TNFα in psoriatic arthritis(PsA) synovium compared to osteoarthritic (OA) or rheumatoid arthritis (RA) samples [42].

Both the psoriatic skin disease and psoriatic arthritis can be evaluated in trials of new therapies. Infliximab was studied in a double-blind, randomized trial of 33 patients

with plaque type psoriasis [43, 44]. As in the ankylosing spondylitis trials, 3 doses of infliximab were infused at weeks 0, 2, and 6. The patient analysis was performed at week 10 and included physician global assessment, National Psoriasis Foundation psoriasis score (NPF-PS), the Psoriasis Area and Severity Index (PASI), and biopsies of normal and involved skin. Responders were rated as good, excellent, or clear on the physician global assessment. Infliximab at 5 mg/kg led to a response in 9 of 11 patients; infliximab 10 mg/kg resulted in a response in 10 of 11 patients; and placebo patients had a response in 2 of 11 cases ($p < 0.01$). In addition, $\geq$ 75% improvement in the PASI occurred in 9/11 (5 mg/kg) and 8/11 (10 mg/kg) versus 2/11 (placebo) patients. Skin biopsies showed statistically significant decreases in epidermal CD3+ cells and thickness only in the infliximab-treated groups. No serious adverse events were reported.

The experience with infliximab for the treatment of psoriatic arthritis consists of open-label trials and the results show benefit through 1 year of therapy [45]. Using etanercept, phase II and phase III randomized, placebo-controlled, double blind trials have been completed. The phase II trial enrolled 60 patients in a comparison of placebo vs etanercept 25 mg sc 2x/week in a 3 month trial with a 6 month open-label extension [46]. Patients had psoriatic arthritis with $\geq$ 3 swollen and tender joints, psoriatic skin disease, prednisone dose of $\leq$ 10 mg/d, and methotrexate at $\leq$ 25 mg/week stable for 2 months. The primary endpoint of the trial was the PsARC (Psoriatic Arthritis Response Criteria). The PsARC requires improvement in at least 2 of these 4 criteria: physician global assessment, patient global assessment, tender joint score, or swollen joint score; improvement in at least 1 of the 2 joint scores; and no worsening in any of the four criteria. Secondary criteria for the phase II etanercept trial were ACR 20, 50, and 70; ACR components; HAQ; target lesion response; and PASI. The placebo and etanercept groups were similar with the etanercept patients' median age 48, 53% male, having had psoriatic arthritis for 9.0 years, having tried 1.5 DMARDs, with 20% on corticosteroids and 47% on methotrexate. The main result showed an 87% PsARC response in the etanercept group and a 23% rate for placebo ($p < 0.001$). The use of methotrexate did not alter the results. Significant improvements were also recorded for the ACR 20 (73% etanercept vs. 13% placebo), ACR 50 (50% vs. 3%), ACR 70 (13% vs. 0%), the tender and swollen joint counts, the HAQ, and the median % improvement of the PASI (46% vs. 9%) and a target skin lesion (50% vs. 0%). During the 6 month open-label follow-up, 50 of the original 60 patients participated. 25% were able to discontinue use of methotrexate and 44% could discontinue steroids.

The phase III trial of etanercept in psoriatic arthritis was a 6 month, double-blind, randomized, placebo-controlled trial at 17 sites in 205 patients [47]. The entry criteria were identical to the phase II trial, and the patient populations were also comparable to the earlier study, with the median age 48, 57% male, duration of psoriatic arthritis 7.1 years, steroid use in 19%, and methotrexate use in 45% of the etanercept

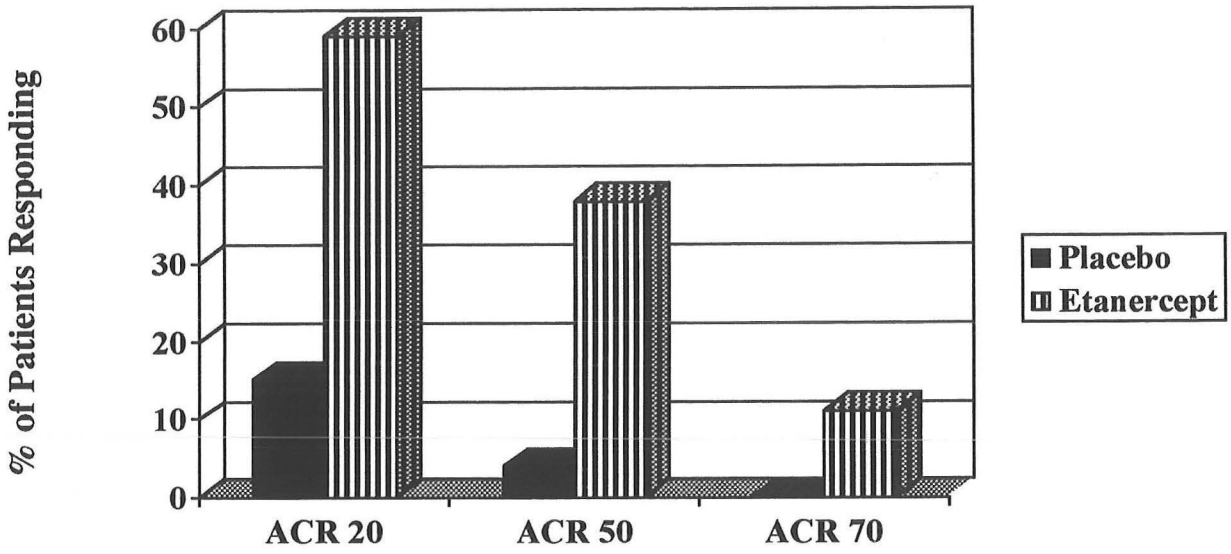

Figure 10. Etanercept in the treatment of psoriatic arthritis. The ACR 20, ACR 50, and ACR 70 responses were all significantly greater in the etanercept-treated patients at 3 months ($p < 0.001$ for all three) [47].

group. The primary endpoint was a different measure, the ACR 20 at 3 months. Secondary endpoints were the ACR 20 at 6 months and the ACR 50 or ACR 70, PsARC, HAQ, SF-36, and for the skin lesions, the target lesion response, the dermatologist's global assessment of target lesion and psoriasis score, and the PASI. The ACR 20, 50, and 70 responses were all highly significant at 12 and 24 weeks. At 12 weeks, the etanercept patients showed 59% response vs. 15% for the placebo group, the ACR 50 was 38% vs. 4%, the ACR 70 11% vs 0% (Figure 10). Again, the PsARC, tender and swollen joint counts, HAQ, target lesion score improvement, and PASI score improvement were all significantly improved in the etanercept group. Overall, both the arthritis and the skin disease improved significantly with etanercept treatment.

IL-1

Although TNFα blockade has substantial benefit in active inflammatory arthritis, as many as 40% of patients will have no or insufficient response. For these patients, other agents are needed. Revisiting the inflammatory cascade that has TNFα at its apex, the cytokine activated immediately downstream of TNFα is IL-1. Experimental evidence indicates that IL-1 does not merely represent a downstream effector in the inflammatory cascade. Instead, it has separate destructive effects not mediated by TNFα and therefore represents a potential therapeutic target.

IL-1 is a 17 kD protein produced by monocytes and macrophages, and also by B cells, activated T cells, and endothelial cells. Two types of cell-surface IL-1 receptors have been described, type I and type II, but only the type I receptors possess a cytoplasmic tail to allow signaling into the cell. The type I receptor is found at low levels on numerous cell types. Both type I and type II receptors are also found as soluble receptor and decrease effective IL-1 levels by binding circulating IL-1. In addition, a naturally-occurring antagonist of IL-1 exists, the IL-1 receptor antagonist (IL-1ra). IL-1ra binds to the type I receptor with high affinity but without activating the receptor, providing a further mechanism of downmodulating IL-1 actions in the body.

The actions of IL-1 include both inflammatory and tissue-remodeling effects [48]. Endothelial cells respond to IL-1 by upregulating adhesion molecules that allow the emigration of leukocytes, as well as releasing PAF (platelet-activating factor), nitric oxide, chemokines (that attract neutrophils, macrophages, and lymphocytes), and prostaglandin E. Lymphocytes stimulated by IL-1 are activated and stimulated to expand. Macrophages respond to IL-1 by activating osteoclast precursors which in turn degrade bone. Similarly, chondrocytes stimulated by IL-1 produce collagenases that are part of cartilage destruction. Fibroblasts and smooth muscle proliferate in response to IL-1, and fibroblasts additionally release inflammatory mediators. Therefore, IL-1 is a powerful proinflammatory molecule that attracts leukocytes and activates them, but also mediates destruction of bone and cartilage.

In animal models of arthritis, manipulation of IL-1 levels has had dramatic effects. Injecting IL-1 into the knee joints of rabbits resulted in degradation of cartilage [49]. On the other hand, injection of antibodies to IL-1 decreased the extent of inflammation and cartilage damage in murine collagen-induced arthritis [50]. Mice with a disrupted IL-1ra gene develop two distinct inflammatory conditions. One group described an inflammatory arthritis beginning at 5 weeks of age, leading to joint deformity and bone erosions similar to rheumatoid arthritis [51]. IL-1 mRNA levels in affected joints were 10 times those of control mice. A second group described a vasculitic phenotype in their mice, characterized by an inflammatory transmural vascular infiltrate of macrophages, neutrophils, and T cells, resulting in arterial stenosis, aneurysms, and hemorrhage [52]. These mice did not develop arthritis. The difference between the strains has been ascribed to unknown effects from the genetic backgrounds, bacterial flora, and exact targeting constructs used by the two groups.

In normal organisms, the inflammatory actions of IL-1 are kept under control by the presence of soluble IL-1 receptors and the IL-1ra. Levels of IL-1ra are elevated in inflammatory arthritis, but not to high enough levels to counteract the inflammatory effects of the even more abundant IL-1. The commercially available IL-1ra (Anakinra, trade name Kineret) is the recombinant human form, a nonglycosylated IL-1ra that differs from the endogenous protein by the N-terminal addition of a methionine. Anakinra binds to IL-1 receptors with the same avidity as endogenous IL-1β and IL-1ra. Its half-life is 4 to 6 hours and is therefore dosed as a daily subcutaneous injection.

The separate actions of TNFα and IL-1 in contributing to an inflammatory arthritis are exemplified by studies of streptococcal cell wall (SCW) arthritis, a rodent model of rheumatoid arthritis. Injection of SCW leads to a rapid early peak of TNFα, and later peaks of IL-1 and the anti-inflammatory IL-10. If TNFα is blocked, there is suppression of paw swelling. Nevertheless, IL-1β is induced normally and late bone erosions typical of a destructive arthritis still occur. Conversely, if IL-1β is blocked, paw swelling is not reduced yet late erosions do not develop. This highlights the differential effects of TNFα and IL-1: TNFα is the dominant cytokine controlling inflammation, while IL-1 is most important in mediating bone destruction. These findings also demonstrate that IL-1 is not merely a part of the TNFα cascade, since IL-1 has independent effects as revealed by blockade of TNFα.

Trials of IL-1ra in rheumatoid arthritis

The initial randomized, double-blind, placebo-controlled study of IL-1ra was a European trial of 472 patients with RA [53]. Patients had active RA, with disease present for 3.7 to 4.3 years, and those on disease-modifying antirheumatic drugs had these washed out for at least 6 weeks. The primary endpoint for the study was an ACR 20 response at 24 weeks. A secondary endpoint was the Larsen score, a radiographic assessment of joint damage. Dosages used in this study consisted of 0, 30, 75, or 150 mg sc qd. The response at 24 weeks was statistically significant only for the highest dose of IL-1ra, with a 43% ACR20 response (vs. 27% for placebo, $p = 0.014$) (Figure 11). The Larsen score showed 41% less deterioration in the IL-1ra groups vs. placebo ($p = 0.03$). In a follow-up study for a further 24 weeks, patients on IL-1ra maintained improvements in number of tender joints, HAQ, ESR, and investigator's assessment, while the placebo patients that were switched to IL-1ra treatment showed clinical improvement similar to the group that received IL-1ra for the original 24 weeks. [54]

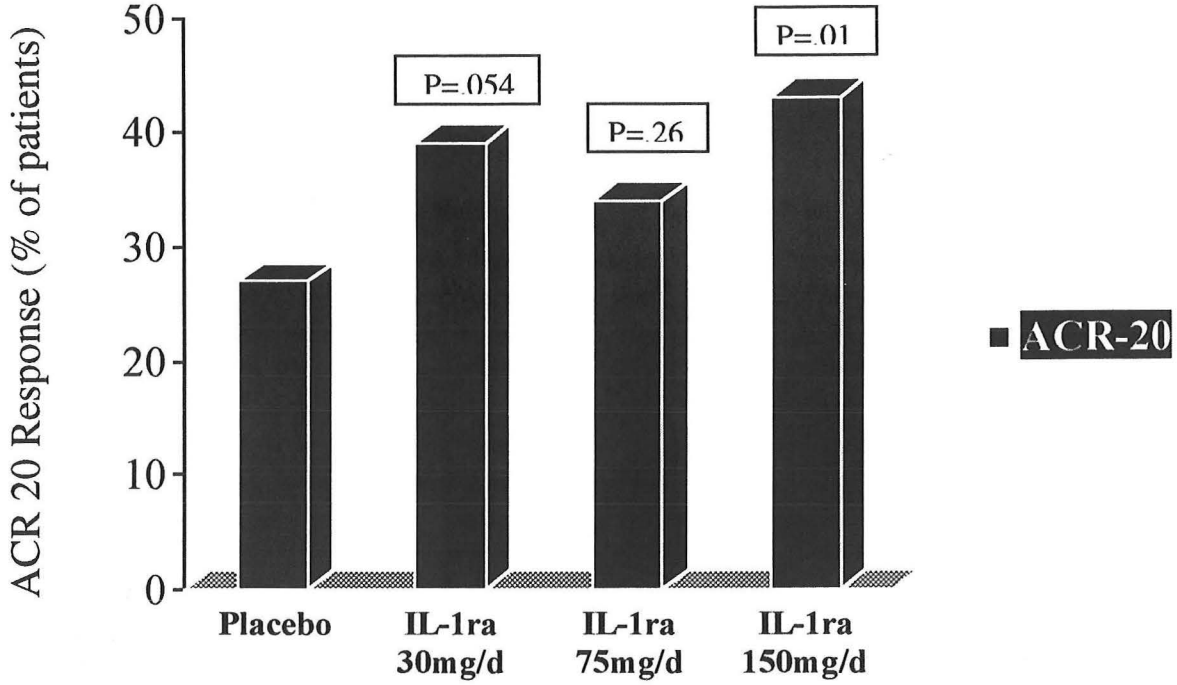

Figure 11. ACR 20 response in a 472-patient trial of IL-1ra. Placebo was compared to 3 different doses of IL-1ra injected sc qd. P values are for a comparison of the IL-1ra group versus the placebo group. The p value for the combined IL-1ra groups vs placebo is 0.020.

A second trial of IL-1ra combined it with methotrexate (Cohen S. et al., Arthritis Rheum, in press). In a randomized, blinded, placebo-controlled trial, 419 patients with active RA were studied for 24 weeks. IL-1ra doses were 0, 0.04, 0.1, 0.4, 1.0, or 2.0 mg/kg sc qd, with background methotrexate at 15-25 mg/week. The primary endpoint was the ACR 20 at 12 weeks, the secondary endpoint the ACR 20 at 24 weeks. The result was a statistically-significant ACR 20 response in the 0.1, 1.0, and 2.0 mg/kg dose group at 12 weeks, and only in the 1.0 mg/kg groups at 24 weeks (Figure 12). The study design using an intention to treat, nonresponder imputation (i.e. assuming that all patients who did not complete the study for whatever reason were nonresponders) is stricter than is used in many other RA clinical trials and likely contributed to the results.

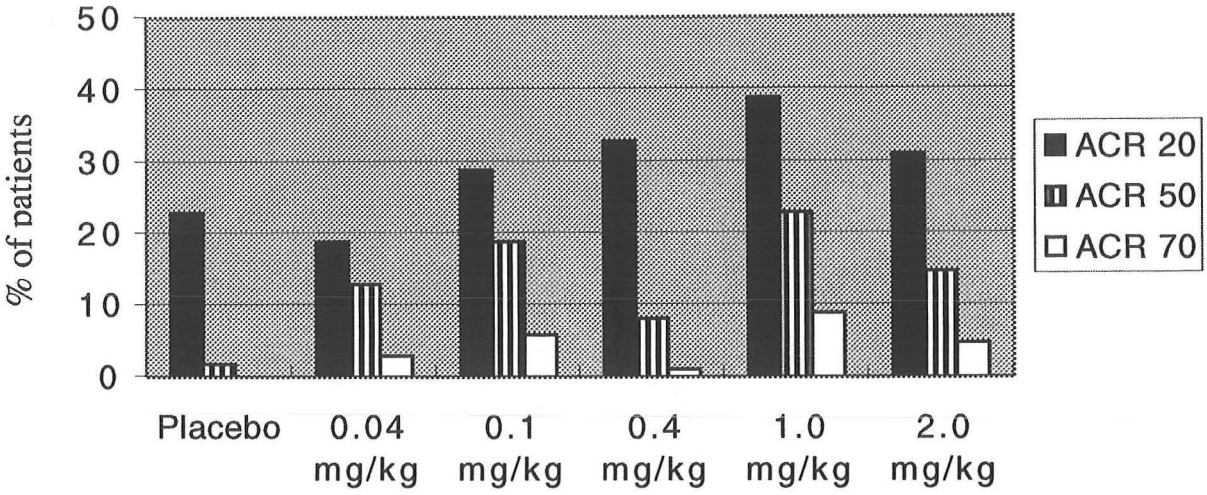

Figure 12. Clinical responses in the methotrexate/IL-1ra combination study. Patients received placebo injections or 5 different doses of IL-1ra sc qd. At week 24, only those receiving an IL-1ra dose of 1.0 mg/kg achieved statistically significant improvement ($p<0.05$).

From previous animal studies, IL-1 antagonism alone did not have as powerful anti-inflammatory effects as TNFα blockade, but did have significant benefits in retarding bone erosion. The trial by Bresnihan et al. described above included measures of hand X ray analysis as measured by Larsen score and by erosive joint counts (Figure 13). Only 74% of the study group had a complete series of X ray available for analysis. The mean Larsen score worsened in the placebo and all IL-1ra groups, but did so 41% less in the combined IL-1ra groups ($p=0.03$; of the individual groups, only the 30mg/d cohort showed statistically significant benefit). For the erosion score, the combined IL-1ra groups had 46% less progression in the number of joints with erosions ($p=0.004$; statistical significance was achieved in the 30 mg/d and the 75 mg/d groups).

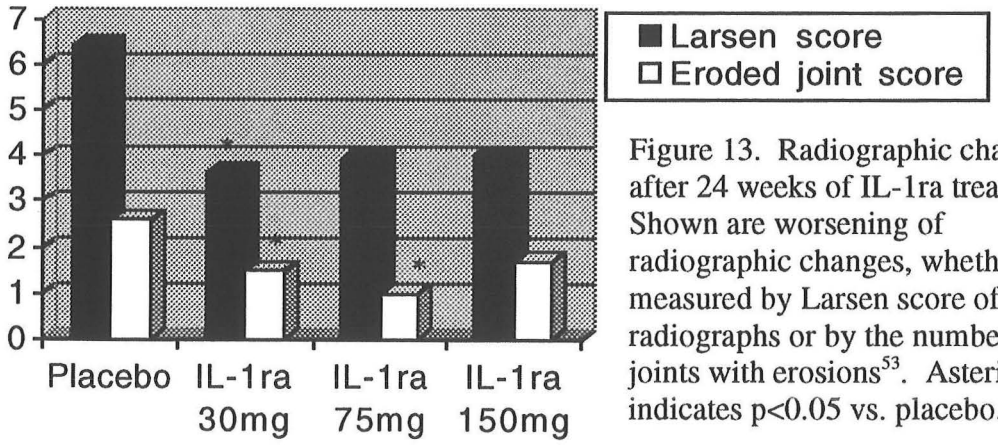

Figure 13. Radiographic change after 24 weeks of IL-1ra treatment. Shown are worsening of radiographic changes, whether measured by Larsen score of hand radiographs or by the number of joints with erosions[53]. Asterisk indicates $p<0.05$ vs. placebo.

A clinically-relevant question is what happens when the IL-1ra is combined with TNFα blockers. This situation will be encountered in those patients with a partial response to TNFα blockade who then need additional therapy. While no controlled studies are available, the combination is being studied for safety first, then for efficacy. Of a series of 58 patients with RA treated with etanercept (25 mg sc b.i.w.) and anakinra (1mg/kg/d sc) for 24 weeks, there were no deaths, 2 pneumonias and 2 episodes of cellulitis. The trend in tender and swollen joints, HAQ score, CRP, and ESR was favorable. Further studies are awaited to assess the true risk of serious infection and the clinical benefit of this combination of drugs.

Conclusions

The etiology of rheumatoid arthritis and other inflammatory arthritides remains unknown but significant progress in treatment of inflammation has been made in the last 3 years. The cytokines TNFα and IL-1 are the first key mediators to be identified as contributing to the proliferation of tissues, the chemotaxis and activation of inflammatory cells, and the destruction of bone and cartilage. Recombinant proteins have been developed as injectable blockers of these cytokines and represent the first of several such products under development. Their success has been demonstrated in controlling the clinical symptoms and findings of inflammatory arthritis, and their success has led to trials in numerous other nonrheumatic inflammatory conditions. For the first time, these drugs also control the progression of bony damage, an effect not demonstrated for previous treatments. In the future, one can expect further trials of agents that block still other inflammatory mediators and/or increase the levels of anti-inflammatory factors. In addition, there is great interest in identifying chemical compounds that act in a similar fashion but can be taken as an oral medication, since the cost and patient acceptance of injected medication remains a concern. There is still room for entirely different approaches, as well, since even the available biologics do not provide relief to all patients and do not "cure" arthritis even in responders, but instead suppress the worst manifestations only for as long as treatment is continued.

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