Is There a Role for TNF-α in Anti-Neutrophil Cytoplasmic Antibody–Associated Vasculitis? Lessons from Other Chronic Inflammatory Diseases

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Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis is the most common cause of rapidly progressive glomerulonephritis and immune-mediated pulmonary renal syndrome. Now that the acute manifestations of the disease generally can be controlled with immunosuppressive drugs, ANCA-associated vasculitis has become a chronic and relapsing inflammatory disorder. The need to develop safer and more effective treatment has led to great interest in the mediators of chronic inflammation. There are many lessons to be learned from studies of other chronic inflammatory diseases, particularly rheumatoid arthritis (RA). The identification of a TNF-α-dependent cytokine cascade in the in vitro cultures of synovium in joints of patients with RA led to studies of TNF blockade in experimental models of arthritis and subsequently to clinical trials. These have culminated in the widespread introduction of anti-TNF therapy not only in RA but also in Crohn disease, ankylosing spondylitis, and several other chronic inflammatory disorders. Following a similar investigative pathway, studies that show the importance of TNF production by leukocytes and intrinsic renal cells in glomerulonephritis have been followed by the demonstration of the effectiveness of TNF blockade in several experimental models of glomerulonephritis and vasculitis. In experimental autoimmune vasculitis, improvement in disease was paralleled by a reduction in leukocyte transmigration, as demonstrated by intravital microscopy. The benefit of infliximab (a mAb to TNF) in ANCA-associated vasculitis was recently reported in a prospective open-label study. However, the use of etanercept (a soluble TNF receptor fusion protein) was not found to be of significant benefit in a randomized, controlled trial in patients with Wegener granulomatosis. Therefore, there is a need for further evaluation of the use of anti-TNF antibodies in patients with ANCA-associated glomerulonephritis.


Cytokines: Protein Mediators of Stress

In the past 20 yr, the importance of the cytokine family of mediators has been uncovered. This had to await the era of molecular biology, because the necessary tools to establish that these highly potent mediators were in fact dominant signals in immunity and inflammation depended on their molecular cloning, with their subsequent production and evaluation in pure form. We now know that there are more than 200 large polypeptides, generically called cytokines, that usually are released from cells to act as short-range mediators of inflammation, immunity, cell mobility, recruitment, fibrosis, and repair (1). Cytokines are known to be important in all biologic processes, from organ development to host defense against pathogenic agents.

The cytokines are described according to their major properties. There are proinflammatory cytokines, anti-inflammatory cytokines, chemokines, and growth factors. Certain families were identified in different ways and so are termed interferons (antiviral proteins), interleukins (acting between leukocytes), or colony-stimulating factors (acting on hemopoietic cell growth). However, all of the colony-stimulating factors also have important functions in inflammation, immunity, and repair and hence are not really distinct. These historical “families” are really misnomers; interleukins also act on other cell types, interferons act on host defense and hemopoietic tissue, and so forth.

In any given acute or chronic inflammatory disease, a wide spectrum of cytokines will be detected in diseased tissue (2). This has been documented most extensively in rheumatoid arthritis (RA), because in this condition, human tissue can be sampled at the height of the disease process without causing injury to the patient, during biopsy procedures early in disease or during joint replacement surgery late in disease (3). This work has provided several important insights into chronic inflammation.

First, in a chronic disease, cytokines that are produced experimentally in a short-term, transient manner are continu-
ously present (3). This observation indicated that the processes in a chronic disease are somewhat different from those studied with short-term stimuli and, not surprising, that chronic disease shows long-term sustained production of cytokines. How these events are related is an interesting question, which is being actively studied.

Second, anti-inflammatory cytokines are upregulated in chronic inflammation as well as proinflammatory cytokines (4). This suggested the concept of a dysregulated equilibrium, as illustrated in Figure 1. This concept was testable in vitro, using cultures of human disease tissue from rheumatoid synovium, by neutralizing the inhibitory cytokines; for example, blocking IL-10 augmented TNF and IL-1 production two-fold (4), and blocking both IL-10 and IL-11 augmented TNF and IL-1 a dramatic five- to 20-fold (5).

Third, there is marked “redundancy” in the cytokine network, which means overlap of biologic properties of the cytokines that are found in the joints in active RA. IL-1, TNF, IL-6, and GM-CSF are all present in signaling quantities in active disease tissue. They do much the same things on target cells. Which are the critical, rate-limiting factors that are good therapeutic targets? This was a major problem, as the new therapeutic approaches of “biotechnology,” making mAb and receptor fusion proteins against specific targets, could be used only experimentally as a single target in clinical trials, so determining which was the best therapeutic target was of critical importance.

**TNF-α Is the Body’s “Fire Alarm”**

Complex biologic systems need to have alarms to detect problems in homeostasis and initiate their resolution. This clearly is the case in multicellular organisms such as human. Stress of any type (e.g., burns, ultraviolet irradiation, x-rays, viruses, bacteria) induces the rapid release and production of TNF-α (6). This acts as the body’s “fire alarm” and induces the rapid arrival of the “firefighters”—leukocytes from the blood. This interpretation is based on several lines of knowledge. The first clue came from the effects of TNF blockade. In human RA cultures in vitro, blocking TNF downregulates all other proinflammatory mediators: cytokines (IL-1, IL-6, IL-8, and GM-CSF), destructive enzymes such as matrix metalloproteinases (MMP), and prostaglandins (3,7). In mice that were given bacteria, a natural challenge to the immune system, TNF is the most rapidly detected cytokine in blood, before IL-1 or IL-6. When anti-TNF antibody is administered, it not only neutralizes the TNF but also diminishes IL-1 and IL-6 levels by four- to five-fold (8). These experiments were reported in 1989 and supported the concept of a TNF-dependent cytokine cascade (Figure 2).

The concept of a TNF-dependent proinflammatory cytokine cascade resolved the dilemma of which cytokine was a potential therapeutic target, but this hypothesis had to be tested in vivo. Animal models of arthritis in the late 1980s had a poor reputation as predictors of clinical success, after the failure of anti-CD4 mAb, which had been effective in mouse models (in the preventive mode) but was not effective in patients with late-stage RA (9). However, the most relevant animal model of human RA, collagen-induced arthritis in DBA/1 mice, was predictive. We consider this model relevant because it demonstrates genetic susceptibility and because these mice develop arthritis spontaneously as they age (10). Furthermore, the MHC genes involved have similar properties in that mouse I-A<sup>a</sup> in the DBA/1 mouse resembles human HLA-DR4 (associated with RA) in peptide selection (11). Hamster anti-mouse TNF antibody that was injected after disease onset was found to ameliorate arthritis and reduce joint damage in this model (12), and this helped to establish the rationale for anti-TNF therapy in RA.

TNF now is recognized as the prototype of a gene superfamly that is important in regulating many biologic functions. Membrane-bound or soluble TNF-α reacts with two receptors, known as TNFR1 (p55) and TNFR2 (p75). These receptors themselves form part of the TNFR superfamily. Research in this field has identified approximately 40 members of the TNF/TNFR superfamilies, and knowledge about their function is rapidly emerging. Detailed consideration of the biology of the TNF/TNFR superfamilies is outside the scope of this article and was reviewed recently (13).
TNF Blockade: Failure and Success

TNF blockade in human medicine has had a checkered career. Evidence that TNF was pivotal in animal models of sepsis (14) led to the concept that TNF blockade would be therapeutic in sepsis, which is the cause of death of approximately 300,000 patients with severe underlying diseases in the United States each year. However, extensive trials of anti-TNF antibodies or TNF receptor fusion proteins in thousands of patients with sepsis were not successful. Benefit was seen in subsets of patients who still had elevated cytokine levels, but in the great majority, there was no benefit and sometimes deterioration (15).

Although this result was unfortunate for the bioscience industry, many lessons were learned from the experience. For the therapy of chronic diseases such as RA, this presented a wonderful opportunity, because specific TNF-inhibiting agents had been produced and tested. When arthritis research identified TNF as a promising therapeutic target, there were multiple antibodies and TNF receptor fusion proteins, available as a legacy from the sepsis work, to be tested in this disease. The clinical trials in RA, initiated first with a chimeric antibody, infliximab, were an instant success (16).

Effective Treatment for RA

Because TNF is an important host defense molecule (6), there was a school of thought that TNF blockade for RA was unethical, because it was too dangerous. Nevertheless, via Jim Woody at Centocor, an open-label clinical trial in RA multidrug treatment failures was initiated (16) with Ravinder Maini and Marc Feldmann as principal investigators. This showed a remarkable response to the chimeric anti-TNF antibody, infliximab (now sold as Remicade). There was rapid onset of benefit in all 20 patients, to a variable degree, but with a median swollen joint reduction of 60 to 70% at 6 to 8 wk. There was very rapid symptomatic benefit, reduction in fatigue within hours, and reduction in stiffness and pain in days, but eventually all patients relapsed by 18 wk. This was amelioration, not a cure (Figure 3).

This trial, with a clear response in an unmet medical need, opened the way to the rapid development of multiple TNF inhibitors, with the introduction of etanercept, a p75 TNFR-Ig fusion protein, being particularly efficient (17). Despite clinical trials on the fusion protein that started after those that used the antibody (infliximab), etanercept obtained Food and Drug Administration approval first. From these studies, much was learned about the way to use anti-TNF therapy, how to minimize immunogenicity (18), and the mechanisms of action (19). This knowledge has been particularly helpful in the development of TNF blockade for other important disease indications in medicine and in understanding how to use most effectively such powerful, expensive, and potentially dangerous therapeutic agents (20) in a safe and cost-effective manner.

The degree of benefit in patients with RA is highly variable. Although few, if any, have no evidence of benefit, many (20 to 40%) do not have a 20% improvement in swollen and tender joints, needed to qualify as American College of Rheumatology responders, depending on the stage of the disease. Late-stage treatment failures, as entered in phase III studies, have a 60% response rate (21), whereas patients in the first month of diagnosis and within 6 mo of the onset of symptoms have a 90% response rate (22). It is important to note that a marked reduction in joint damage occurs in the majority, and in a substantial proportion, there is evidence of bone and cartilage repair (21,23). The important mechanistic insights from these studies have been to verify that the TNF-dependent cytokine cascade that is detected in rheumatoid synovial cultures in vitro is operative in patients with RA in vivo (24) and to show that TNF blockade diminishes leukocyte recruitment (25).

There have been extensive studies of how anti-TNF therapy mediates clinical benefit in RA. Multiple cytokines are rapidly downregulated in vivo, within a few hours, indicating a direct effect of TNF inhibition. Effects that are measured at a few weeks may be very indirect. IL-6 levels in blood are rapidly reduced, as are IL-1, IL-8, monocyte chemoattractant protein-1, and vascular endothelial growth factor. Cytokine levels in joints also are diminished in biopsy samples, including IL-1 and IL-6. Acute-phase protein levels, including C-reactive protein, serum amyloid A (SAA), and fibrinogen, are reduced. There also are rapid changes in hematologic indices. Elevated levels of granulocytes and monocytes are reduced, low hemoglobin is restored, and high platelet levels normalize. Lymphocyte changes are complex. There are more IFN-γ-producing cells in blood, diminished mitogen and recall responses normalize, and poorly functioning regulatory cells are restored. Elevated serum levels of MMP (e.g., MMP-1, MMP-3) normalize, providing...
a partial reason for reduced joint damage. Synovial cellularity is diminished within 2 to 4 wk, probably as a result of reduced influx of inflammatory cells, but there are no data on changes in egress. Possible changes in apoptosis are controversial. These studies have been reviewed thoroughly (19,23–26).

With the extensive improvement in the acute-phase response, including reductions in atherosclerotic risk factors such as fibrinogen, high platelets, and high C-reactive protein, it was of interest to ascertain whether cardiac complications, augmented in RA, would diminish. This was indeed the case for both heart failure (27) and atherosclerotic complications (28). The side effects of anti-TNF therapy are relatively modest, and there is no doubt that the benefits outweigh the risks (29,30). Infection is the most predictable, because a major host defense molecule is compromised, but numbers are comparatively low. The most common opportunistic infection is tuberculosis, with the risk for infection augmented four-fold, if precautions (screening, etc) are not taken (20). With screening now routine and prophylaxis given if in doubt, this problem is dramatically reduced. Other opportunistic infections are much more rare. There still is debate about the risk for respiratory infections, such as pneumonia. A survey of fewer than 1000 German patients suggested that it was augmented two-fold, but a larger British survey so far has failed to show an increased risk for pneumonia.

Initially, there was a concern about increased numbers of lymphomas in anti-TNF–treated patients, despite the fact that it was known that the risk for lymphomas in RA was proportional to duration and disease activity. Whole population databases in Sweden have clarified that there is in fact no increased risk (31). It long has been recognized that anti-TNF–treated patients have no overall increased risk for cancer.

Role of TNF Blockade in Other Chronic Inflammatory Diseases

With the success of TNF blockade in RA, as a result of downregulation of the proinflammatory cytokine cascade in the synovium of the inflamed joint, this approach subsequently was tested in other chronic diseases in which TNF was implicated (25). The first success was in Crohn disease; patients who had steroid-resistant Crohn disease and were in flares responded well. Not only were the symptoms relieved, as judged by the Crohn disease activity index (32), but also the major complication of fistula formation responded in the majority of cases (33). Infliximab therefore was approved for short-term use in Crohn disease in 1998, and subsequent trials have permitted long-term use. Trials in other diseases then were initiated, and there currently is approval in six: RA, Crohn disease, juvenile RA, anklyosing spondylitis (34), psoriasis (35), and psoriatic arthritis (36).

Successful trials and case studies now have been reported in many other diseases, as listed in Table 1, but there also have been interesting and intriguing failures. Etanercept, just as effective as infliximab in RA, is not effective in Crohn disease (37,38). Various hypotheses may be suggested for this—an inadequate dose is one, and a lack of apoptosis induction is another—but none is established. It is interesting that adalimumab, a fully human anti-TNF antibody, also is effective in Crohn disease (39). Lenenercept, a p55 TNFR-Ig fusion protein, was tested in multiple sclerosis but was not successful (40), probably because it did not penetrate the central nervous system. More recently, a short-term trial of infliximab in chronic obstructive pulmonary disease (41), was not successful, but a trial in severe steroid-resistant asthma showed benefit (42).

<table>
<thead>
<tr>
<th>Disease</th>
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<tr>
<td>Rheumatoid arthritis, juvenile rheumatoid arthritis, Crohn disease, psoriatic arthritis, ankylosing spondylitis, psoriasis</td>
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<td>Trials not completed and pilot studies</td>
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<td>Ulcerative colitis, Behçet syndrome, vasculitis (small and large vessel), glomerulonephritis, SLE, joint prosthesis loosening, hepatitis, polymyositis, systemic sclerosis, amyloidosis, sarcoidosis, ovarian cancer, steroid-resistant asthma, refractory uveitis</td>
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<tr>
<td>Clinical failures</td>
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<td>Congestive heart failure, multiple sclerosis, COPD, Sjögren syndrome, Wegener granulomatosis (etanercept)</td>
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Are There Therapeutic Applications for Small-Molecule Drugs that Can Mimic the Efficacy of TNF Blockade?

With the medical and commercial success of TNF blockade with antibodies or TNF receptor fusion proteins, many pharmaceutical companies have started to work on drugs to block TNF. The most popular approach has been to try to block p38 mitogen-activated protein kinase (43). This is involved in mRNA stability and translational efficiency (44), and p38 blockade markedly reduces TNF production in vivo in animal models, as well as reducing TNF signaling. This target has been the subject of considerable research, but so far the drugs studied in trials have been too toxic for routine clinical use. However, there still are numerous active drug discovery programs, and this approach may yet be successful. As an alternative, NF-κB blockade would provide inhibition both of TNF production (although not in all circumstances) and of TNF signaling. Attempts to develop drugs that block the inducers of NF-κB kinase (IKK), especially IKK2, have not yet been successful, although work still is in progress (45).

Several alternative approaches may be safer than the above and so may reach clinical use sooner. Some possibilities that we have examined include phosphodiesterase type 4 (PDE4) inhibition (46), nonpsychoactive cannabinoids, and blockade of the Bruton tyrosine kinase (btk)/tec tyrosine kinases (47). PDE4 enzymes regulate cAMP in both T cells and macrophages, and this leads to reduced production of both T cell cytokines (IL-12

Table 1. TNF is a good target in many chronic inflammatory diseasesa
and IFN-γ) and macrophage cytokines (TNF). In animal models, PDE4 blockade is effective in arthritis (46), but in humans, problems with nausea and emesis have been limiting. Newer compounds that lack these adverse effects may prove effective in future. Cannabinoids have anti-immune and anti-inflammatory properties. Cannabidiol is a nonpsychoactive cannabinoid, without significant capacity to bind to CB1 or CB2 receptors, which in vitro is inactive but which in vivo is effective in animal models of RA, multiple sclerosis, and Crohn disease (48). Cytokine blockade is demonstrable in tissues that are taken from the treated animals; therefore, this family of drugs is a potential therapeutic candidate. A surprise in RA treatment in recent years has been the considerable benefit of the anti-CD20 antibody rituximab, which kills mature B cells (49) but not plasma cells. btk is defective in X-linked agammaglobulinemia, in which B cells do not mature. btk also is important in TNF production, so btk and its close relative tec are interesting targets, as their inhibition would block two pathways of importance in RA: B cells and TNF (47).

Involvement of TNF-α in Renal Inflammation

As in the other chronic inflammatory diseases described above, there now is considerable evidence that TNF-α plays an important role in glomerular inflammation and scarring. TNF production has been demonstrated within the glomeruli in both experimental and human glomerulonephritis (50), including that associated with anti-neutrophil cytoplasmic antibody (ANCA) (51). Although it is clear that infiltrating macrophages are an important source of TNF, several intrinsic renal cell types (including mesangial cells and epithelial cells) also contribute. Experiments in the late 1980s revealed that systemic administration of TNF was able to induce glomerular damage in normal rabbits (52). More interesting, a small dose of TNF greatly increased glomerular damage in rats with nephrotoxic nephritis that was induced by administration of rabbit anti-rat glomerular basement membrane antibodies (nephrotoxic serum) (53).

TNF Blockade in Experimental Glomerulonephritis

Several studies now have confirmed the importance of TNF-α in glomerular inflammation by using a variety of blocking strategies. TNF-binding protein, a dimeric form of the soluble receptor, reduced renal injury in rat nephrotoxic nephritis and also was found to decrease serum levels of macrophage migration inhibitory factor (MIF) (54). A soluble fragment of the extracellular domain of TNFR1 (p55 TNFR-Ig) was effective in reducing glomerular injury in a short-term model of LPS-enhanced nephrotoxic nephritis. This was accompanied by reduction in glomerular IL-1β expression (55). Both of these experiments suggest that TNF blockade can modulate production of other “downstream” proinflammatory cytokines, which may contribute to the therapeutic effect.

More recent studies in our laboratory examined the effect of TNF-α blockade in both prevention and treatment of nephrotoxic nephritis in the WKY rat. This strain is particularly susceptible to glomerular injury and consistently develops severe crescentic nephritis within 2 wk of the administration of nephrotoxic serum. This is followed by glomerular and interstitial scarring, with renal impairment, by 4 wk (56). Soluble p55 TNFR-Ig was of benefit in both prevention and treatment of the early stage of the disease (57). A mAb to rat TNF-α also was effective in the treatment of established disease because, when started at day 4 (maximum glomerular hypercellularity), there was a marked reduction in crescent formation and improvement in renal function by day 14. This effect was sustained to day 28, when there was significantly less renal scarring in treated animals (Figure 4). It is interesting that when treatment was started at the peak of crescent formation at day 14 and continued until day 28, there still was a significant reduction in tubulointerstitial scarring and preservation of renal function (58). This work suggests that TNF-α is important not only in the
acute inflammatory response but also in the subsequent renal fibrosis. As an alternative approach to TNF blockade, we have examined the effect of rolipram, an inhibitor of PDE4 (as discussed above). Rolipram was effective in both prevention and treatment of nephrotoxic nephritis, and this was associated with a reduction in renal production of TNF-α (59).

The effects of TNF-α in glomerular inflammation and scarring also have been investigated using various knockout mice. TNF-α knockout mice showed a reduction in crescent formation in a model of nephrotoxic nephritis (60). Bone marrow transplantation has been used to create chimeric mice to distinguish the role of bone marrow–derived versus intrinsic renal cell production of TNF. The work of Tipping and colleagues (61) demonstrates that intrinsic renal cells are the major source of TNF-α contributing to renal injury in murine crescentic glomerulonephritis. The role of TNF in renal fibrosis has been examined in the model of unilateral ureteric obstruction. Mice that were deficient in either TNFR1 or TNFR2 showed a reduction in interstitial scarring, as compared with wild-type mice, although deficiency of TNFR1 seemed to have a greater effect (62).

Most recently, the role of TNF was investigated in rodent models of ANCA-associated vasculitis. The model described by Jennette’s group is induced by transfer of anti-myeloperoxidase (anti-MPO) antibodies raised in MPO knockout mice to naive recipients. This results in development of pauci-immune necrotizing glomerulonephritis in the recipient mice (63). A further study showed that the severity of renal injury that was induced by anti-MPO antibodies could be enhanced by administration of LPS, a finding that is consistent with the observation that intercurrent infection can induce relapse in patients with systemic vasculitis. The administration of LPS transiently induced circulating TNF, and a mAb to TNF attenuated the severity of glomerular injury (64). In a different mouse model, involving the transfer of anti–proteinase 3 (anti-PR3) antibodies raised in PR3-deficient mice, intradermal injection of TNF-α triggered local inflammation. This work demonstrates that an additional stimulus, such as TNF-α, is required for the pathogenic effects of ANCA (65).

We have developed a different model of systemic vasculitis by immunizing WKY rats with MPO. These animals develop circulating anti-MPO antibodies, accompanied by a pauci-immune crescentic nephritis and alveolar hemorrhage (66). This has been termed experimental autoimmune vasculitis (EAV) and is effectively a model of ANCA-associated microscopic polyangiitis. Using intravital microscopy, we have demonstrated that anti-MPO antibodies in EAV are capable of inducing leukocyte adhesion and transmigration in vivo and furthermore that they can induce microvascular hemorrhage. The administration of a blocking mAb to TNF in this model virtually abolishes crescent formation and reduces lung hemorrhage. These beneficial effects are accompanied by a reduction in leukocyte transmigration, as demonstrated by intravital microscopy (Figure 5) (67). This work, in an autoimmune model that is highly relevant to human systemic vasculitis, further illustrates the importance of TNF in recruitment of leukocytes to inflammatory sites.

**Figure 5.** Delayed treatment with anti-TNF antibody in experimental autoimmune vasculitis. (A) Severity of focal proliferative glomerulonephritis. Δ, anti-TNF antibody; ■, controls. (B) Transmigration of leukocytes assessed using intravital microscopy. Δ, controls; ■, anti-TNF antibody. Adapted from reference (67). Illustration by Josh Gramling—Gramling Medical Illustration.

**TNF Blockade in ANCA-Associated Glomerulonephritis**

Current management of ANCA-associated systemic vasculitis and focal necrotizing glomerulonephritis is based on the use of prednisolone and cyclophosphamide (68–70). However, the regimens used carry considerable drug-related adverse effects and are associated with a high incidence of relapse. New, more effective, and less toxic approaches to treatment clearly are needed (71). Because of the beneficial effects of TNF blockade in other chronic inflammatory disorders and because of the evidence supporting the role of TNF in experimental glomerulonephritis, it was logical to attempt this approach in human glomerulonephritis. Several small pilot studies suggested a benefit of anti-TNF antibody (infliximab) (72–74) or soluble p75 receptor (etanercept) (75) in ANCA-associated systemic vasculitis.

A larger, prospective, open-label study of infliximab, involving 32 patients with biopsy-proven ANCA-associated vasculitis (both Wegener granulomatosis and microscopic polyangiitis), was reported recently (76). Two groups of patients were studied: (1) Those who presented with acute disease and (2) those with persistent or “grumbling” disease despite immunosuppressive treatment. In group 1, anti-TNF antibodies were used in addition to conventional therapy, but in group 2, the addition of anti-TNF antibodies was the only change in therapy. In
both groups, there was a rapid response in the great majority (88%) of patients at a mean time of 6.4 wk (Figure 6). Although it is difficult to interpret the additional effect of infliximab in group 1 patients, there was a rapid clinical response and a significant steroid-sparing effect when compared with standard regimens. In group 2 patients, in which infliximab was the only change in therapy, the results suggest that TNF-α blockade was responsible for the observed remissions. However, it should be noted that relapse occurred in 18% of patients and that adverse events, particularly infection, were seen in 21% of patients.

A randomized, controlled trial of the use of etanercept in Wegener granulomatosis was published recently by the Wegener Granulomatosis Etanercept Trial (WGET) Research Group (77). In this study, patients with active Wegener granulomatosis were randomly assigned to receive standard therapy, together with either etanercept 25 mg twice weekly or placebo. Of 174 patients analyzed, 72% developed sustained remission, but there was no significant difference between the etanercept and control arms. Disease flares were common in both arms and were not significantly different according to treatment (118 etanercept, 134 control). However, only approximately 50% of cases in both arms had evidence of renal involvement, with a mean serum creatinine of 1.85 mg/dl in the etanercept group and 1.62 mg/dl in the control group. Because renal disease was not analyzed separately, it is difficult to comment directly on the effect of etanercept on ANCA-associated glomerulonephritis. It is notable that >50% of patients in both groups experienced at least one severe adverse event. Six solid cancers were noted in the etanercept group, although it was reported that a higher proportion of these patients had a history of failed treatment before enrollment.

It is not appropriate to make direct comparisons between the results that were obtained in the open-label study of infliximab and the much larger randomized, controlled trial of etanercept. However, these are different approaches to treatment, used in different patient groups, so it is appropriate to consider reasons for the apparently contrasting results. First, it is possible that the dose of etanercept that was used in WGET was not sufficient. Second, it may be that etanercept is less effective in granulomatous disease, which was a major clinical feature in WGET; similar findings have been reported in Crohn disease and sarcoidosis. Third, there are important biologic differences in the effects of etanercept and infliximab; for example, etanercept also binds to LTα and infliximab can bind directly to cell membrane–expressed TNF and induce apoptosis in activated cells in vitro. Whether this effect is important in vivo is controversial, as apoptosis noted during therapy of Crohn disease with infliximab correlates with clinical success, but this is not seen in RA. In considering future trials of TNF blockade in vasculitis, thought also should be given to the high incidence of adverse events in these studies and whether anti-TNF therapy might allow a reduction in the use of other immunosuppressive agents.

**Conclusion**

We have reviewed the role of TNF-α in chronic inflammation and described how TNF blockade was taken from human *in vitro* systems to murine models of arthritis *in vivo* and then into clinical practice in patients with RA. The use of anti-TNF therapy now is approved in RA and several other chronic inflammatory disorders. In renal disease, preclinical studies in experimental models have revealed clearly an important role for TNF-α in glomerular inflammation. In certain mouse models of glomerulonephritis, there is a major role for intrinsic renal cell production of TNF-α, as opposed to that produced by infiltrating leukocytes. Blocking mAb to TNF has proved to be effective in several models of crescentic nephritis in rats, and the benefit in EAV was linked to reduced leukocyte transmigration *in vivo*. Although there are encouraging reports of the use of infliximab in open-label studies of patients with systemic vasculitis, enthusiasm for this approach must be tempered by the lack of effectiveness of etanercept in a controlled trial of patients with Wegener granulomatosis. Overall, we believe that mAb to TNF-α may have a role in the treatment of ANCA-associated glomerulonephritis and suggest that this approach be further evaluated.
Acknowledgments

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References

mokine levels and leukocyte traffic to joints by tumor necrosis factor alpha blockade in patients with rheumatoid arthritis. *Arthritis Rheum* 43: 38–47, 2000
40. The Lenenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group: TNF neutralization in MS: Results of a randomized, placebo-controlled multicenter study. *Neurology* 53: 457–465, 1999
mediated glomerular injury in vivo by IL-1ra, soluble IL-1 receptor, and soluble TNF receptor. *Kidney Int* 48: 1738–1746, 1995


63. Pfister H, Ollert M, Frohlich LF, Quintanilla-Martinez L, Colby TV, Specks U, Jenne DE: Antineutrophil cytoplasmic autoantibodies against the murine homolog of proteinase 3 (Wegener autoantigen) are pathogenic in vivo. *Blood* 104: 1411–1418, 2004


