TNF-α Bioactivity-Inhibiting Therapy in ANCA-Associated Vasculitis: Clinical and Experimental Considerations

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Wegener's granulomatosis, microscopic polyangiitis, idiopathic necrotizing crescentic glomerulonephritis, and Churg-Strauss syndrome are associated with the presence of ANCA with specificity for myeloperoxidase or proteinase 3. Current therapy consists mainly of corticosteroids and cyclophosphamide, but because this treatment regimen is associated with considerable morbidity, other treatment modalities remain desirable. There is compelling evidence that TNF-α plays an important role in the pathogenesis of ANCA-associated vasculitis. Consequently, inhibition of TNF-α bioactivity potentially results in attenuation of disease. This review discusses whether TNF-α bioactivity-inhibiting drugs are useful in the treatment of ANCA-associated vasculitis. The results of *in vitro* and *in vivo* experiments, as well as clinical studies, are evaluated. Although the importance of TNF-α during lesion development is evident, clinical trials that use TNF-α blockers in patients with ANCA-associated vasculitis give mixed results. Importantly, in a large-scale, randomized trial, treatment with etanercept was found not to be effective and resulted in an excess of treatment-related morbidity. It remains to be investigated whether inhibition of TNF-α bioactivity is effective in a subgroup of patients.

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Wegener's granulomatosis (WG), microscopic polyangiitis, idiopathic necrotizing crescentic glomerulonephritis (NCGN), and Churg-Strauss syndrome are associated with anti-neutrophil cytoplasmic autoantibodies (ANCA) (1). They are widely used as serum markers not only for the diagnosis of systemic vasculitis but also for follow-up, because ANCA levels predict disease reactivation in most patients (2). In vasculitis and glomerulonephritis, the main ANCA antigens are the myeloid enzymes myeloperoxidase (MPO) and proteinase 3 (PR3), but in a minority of patients, ANCA are specific for other neutrophil proteins, such as elastase.

To date, therapy for ANCA-associated disease consists mainly of immunosuppression with high-dose glucocorticoids and cyclophosphamide during the induction phase and azathioprine in combination with low-dosage steroids as maintenance therapy. This therapeutic regimen is associated with considerable morbidity and often proves to be insufficient for the induction of a sustained remission, because most patients develop relapsing disease activity during follow-up. Consequently, renal, pulmonary, and/or other organ damage is encountered (3).

In various immune-mediated diseases, including rheumatoid arthritis (4–6), Crohn's disease (7), and ankylosing spondylitis (8,9), the efficacy of TNF-α–inhibiting treatment is well established. Also in several forms of vasculitis, such as Takayasu arteritis (10) and Behçet's disease (11–13), there is evidence that inhibition of TNF-α bioactivity is beneficial.

Here, we review the literature on the role of TNF-α in ANCA-associated vasculitides. *In vitro* studies as well as *in vivo* experimental data and clinical trials on the feasibility of TNF-α bioactivity inhibition are discussed, as well as their consequences for the future use of this treatment modality in patients with ANCA-associated disease.

Role of TNF-α in ANCA-Associated Vasculitis: *In Vitro* Observations

A considerable amount of data that were obtained from *in vitro* experiments point toward a role for TNF-α in the pathogenesis of ANCA-associated disease. Importantly, ANCA-induced neutrophil activation is greatly enhanced by TNF-α, leading to an increased release of oxygen radicals and toxic granule constituents (14–18). The exact mechanism of this effect is uncertain, but several reports demonstrate an increased presence of MPO and/or PR3 on the outer membrane of neutrophils after incubation with TNF-α (16,17,19,20). This increases the availability of the ANCA antigens for binding of the autoantibodies. In addition, neutrophil priming with TNF-α causes upregulation of various molecules that are involved in adhesion of neutrophils to the endothelium (21), and it has been demonstrated convincingly that one of these molecules in particular, the β_2 integrin CD11b/CD18 (CR3), is critically involved in ANCA-induced neutrophil activation (22). *In vitro*, TNF-α pretreatment of endothelial cells makes them more susceptible to damage that is induced by incubation with ANCA-stimulated neutrophils, in particular when those neutrophils

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also are preincubated with TNF-α (23,24). Moreover, pretreatment of human umbilical cord vascular endothelial cells with TNF-α is necessary for the establishment of firm adhesion of ANCA-stimulated neutrophils to those cells (25).

Taken together, *in vitro* data point toward a mechanism in which TNF-α and ANCA together induce the activation of neutrophils. These activated neutrophils attach to the endothelium, release their toxic granule constituents and oxygen radicals, and thereby cause vascular damage.

TNF-α in Animal Models of Autoimmune Crescentic Glomerulonephritis

In crescentic glomerulonephritis, the role of TNF-α has been investigated most thoroughly in animal models of anti–glomerular basement membrane (GBM) glomerulonephritis. In these models, heterologous antibodies to the GBM are administered to mice or rats, respectively, either with (in the accelerated model) or without (in the heterologous model) preceding immunization with unspecific heterologous antibodies. This results in crescentic glomerulonephritis that is characterized by massive early neutrophil influx and an abundance of immune complexes.

Le Hir *et al.* (26) studied the role of TNF-α in the accelerated model of anti-GBM glomerulonephritis using TNF-α knockout (TNF-$\alpha^{-/-}$) mice and found that TNF-α deficiency led to attenuation but no complete inhibition of disease as reflected by delayed onset of proteinuria and attenuation of histopathologic and immunohistochemical alterations. Importantly, no significant decrease in early neutrophil influx could be observed in the TNF-$\alpha^{-/-}$ mice.

Whereas TNF-$\alpha^{-/-}$ mice are only partially protected from disease in the accelerated model of anti-GBM GN, treatment with daily doses of a soluble TNF-α receptor (sTNFr) from day −1 onward completely prevents the development of crescents in the heterologous rat model of anti-GBM GN (27). An explanation for the discrepancy between this study and the study in TNF-$\alpha^{-/-}$ mice by le Hir *et al.* is not provided, but it is conceivable that TNF-α plays a more profound role in the heterologous, neutrophil-dependent, than in the accelerated model of anti-GBM disease. Alternatively, TNF-$\alpha^{-/-}$ mice, because of their lifelong lack of endogenous TNF-α, may have developed a compensatory mechanism for this deficiency, thereby increasing the levels of alternative proinflammatory cytokines that play a role in the development of anti-GBM GN. It is interesting that in the rat model, treatment of established anti-GBM disease (from day +4 onward) with sTNFr resulted in a marked attenuation of disease 10 d after disease induction, reflected by reduced proteinuria, crescent formation, fibrinoid necrosis, and glomerular influx of monocytes and cytotoxic T cells (27). This suggests that TNF-α plays a role not only in the initial phase of disease development but also in maintaining the disease, a notion that is supported further by a more recent study from the same group, in which the effect of a TNF-α–inhibiting mAb on the course of (heterologous) anti-GBM GN was studied in rats that were followed for as long as 28 d (28). In this study, treatment of established anti-GBM GN with anti–TNF-α mAb from day 4 or day 14 until the rats were killed at day 28 significantly attenuated disease as measured by glomerular and tubulointerstitial scarring and serum creatinine level.

Investigation of the role of TNF-α in ANCA-associated GN long has been hampered by the lack of an animal model in which the pathogenicity of ANCA could be demonstrated convincingly. In a mouse model of spontaneous crescentic glomerulonephritis in SCG/Kj mice (29), TNF-α levels were shown to be elevated compared with healthy C57Bl/6 mice (30). Although MPO-ANCA are detected in these mice, they also display massive glomerular immune complex depositions and elevated titers of anti-nuclear autoantibodies (31). Therefore, the contribution of MPO-ANCA to the pathology that is observed in this model is questionable.

An alternative mouse model of MPO-ANCA–associated vasculitis is provided by Xiao *et al.* (32), who showed that transfer of IgG or splenocytes from murine MPO-immunized MPO knockout (MPO$^{-/-}$) mice to wild-type or immune-deficient mice induces disease manifestations similar to those observed in human MPO-ANCA–associated disease. Using the passive transfer model of anti-MPO IgG-induced glomerulonephritis, we recently demonstrated that a "second hit" with LPS significantly increased the severity of anti-MPO IgG-induced lesions (33). Furthermore, in this accelerated model of MPO-ANCA–induced GN, we found that administration of a single dose of TNF-α bioactivity-inhibiting mAb 1 d before disease induction led to a significant decrease but no complete inhibition of renal disease as measured by the degree of urinary abnormalities and the percentage of crescentic glomeruli. This study suggests that TNF-α plays an important, although not pivotal, role in the pathogenesis of NCGN induced by anti-MPO IgG and LPS. However, the fact that these results are obtained after preemptive anti–TNF-α treatment might limit their applicability for the clinical situation.

It is interesting that the effect of TNF-α bioactivity-inhibiting therapy also was investigated recently in a novel rat model of MPO-ANCA–associated NCGN. In this model, the immunization of WKY/NCrlBR rats with human MPO leads to the generation of anti-human MPO antibodies that cross-react with rat MPO, resulting in pulmonary vasculitis and pauci-immune NCGN in some animals (34). In addition, treatment of established disease with a TNF-α–inhibiting mAb strongly reduced albuminuria and completely reversed crescent formation (35).

Taken together, data obtained from animal models of anti-GBM as well as MPO-ANCA–associated NCGN clearly indicate that TNF-α plays an important role in disease induction and progression and suggest a beneficial role for TNF-α bioactivity inhibition in humans. Due caution should be taken, however, when extrapolating the results from animal experiments to the human situation.

TNF-α Inhibition: Human Studies

The efficacy of two TNF-α–inhibiting drugs has been investigated in patients with ANCA-associated NCGN. First, etanercept (Enbrel, Wyeth Pharmaceuticals, Madison, NJ) is a fusion protein of two p75 subunits of the TNF-α receptor, linked to the Fc portion of human IgG1. Second, infliximab (Remicade, Centocor, Horsham, PA) is a chimeric IgG1 mAb that binds and

inhibits soluble as well as membrane-bound TNF-α. The efficacy of both treatments in patients with rheumatoid arthritis seems to be comparable (36), but in patients with Crohn's disease, etanercept, in contrast to infliximab, seems not to be effective (37). The role of adalimumab (Humira, Abbott, Chicago, IL), a novel, fully human TNF-α–inhibiting mAb (38) that has been shown to be effective in rheumatoid arthritis and Crohn's disease when the response to etanercept or infliximab is lost (39,40), in the treatment of ANCA-associated vasculitis remains to be established.

From the *in vitro* and *in vivo* studies on the role of TNF-α in ANCA-associated vasculitis discussed above, it may be hypothesized that an effect of anti–TNF-α treatment would be the result of inhibition of TNF-α–induced preactivation of neutrophils. This would lead to decreased membrane expression of the ANCA antigens, which makes it impossible for the autoantibodies to exert their pathogenic effect.

Several clinical studies provide indirect evidence for a role of TNF-α in ANCA-associated vasculitis. First, plasma levels of TNF-α are increased in patients with ANCA-associated GN, and increased amounts of TNF-α can be found by immunohistochemistry (41). Second, the fractional excretion of TNF-α is increased in patients with ANCA-associated GN, suggesting local production by renal cells or infiltrating leukocytes (42). Third, response to treatment with intravenous Ig is accompanied by a decrease in serum TNF-α level (43). Finally, it has been shown in rheumatoid arthritis that TNF-α is pivotally involved in a tightly regulated network of proinflammatory cytokines and is responsible for the increased production of IL-1, IL-6, and IL-8 and GM-CSF (reviewed in reference [44]). Consequently, inhibition of TNF-α bioactivity in patients with ANCA-associated vasculitis also would result in decreased levels of those cytokines and thereby decrease vascular inflammation.

The human studies on TNF-α bioinhibition in ANCA-associated vasculitis are summarized briefly in Table 1. In the first study, infliximab was added in an uncontrolled manner to standard treatment that consisted of corticosteroids and cyclophosphamide in six patients with treatment-resistant WG (45). In five cases, this led to remission that lasted for 6 to 24 mo. Addition of infliximab to standard immunosuppressive therapy also led to remission in a more recently published case of Wegener's disease (46).

A large, prospective, but also uncontrolled trial among patients with MPO- or PR3-ANCA–positive vasculitis was published in 2004 (47). Addition of infliximab to standard therapy at initial presentation, during relapse, or during persistent disease activity was followed by a clinical response in 28 of 32 patients, as measured with the Birmingham Vasculitis Activity Score (48) and reflected by a decrease in C-reactive protein, serum creatinine, and required steroid dosage. Unfortunately, however, many adverse effects were observed, including several cases of infection, thrombotic events, and a case of B cell lymphoma, complicating clinical response. In addition, despite ongoing treatment with infliximab, relapses were observed frequently. This suggests an escape mechanism that might be mediated partly by the development of anti-infliximab antibodies (6,49–52), although this was not investigated in detail. In an accompanying paper, the administration of infliximab significantly improved endothelial function in 10 patients with active ANCA-associated disease, as measured by endothelium-dependent vasodilation, and also in these patients, a clinical response was observed (53).

Until now, studies have focused mainly on the addition of infliximab to standard immunosuppressive therapy, and there is only limited information on infliximab replacing standard treatment. In an uncontrolled study on 10 patients with conventional therapy-resistant systemic vasculitis, seven of whom had WG, Bartolucci *et al.* (54) found that treatment with corticosteroids and infliximab led to a considerable resolution of disease symptoms and corticosteroid requirement at 42 d and 6 mo. Although limited in size and uncontrolled, this study encourages further research into the replacement of conventional treatment by infliximab in individuals who have ANCA-associated vasculitis and fail to enter remission under standard therapy. A case report in which remission induction was

Table 1. Human studies on TNF-α inhibition[a]

Reference	Study Type	*n*	Diagnosis (*n*)	Intervention	Remission (*n*)	Follow-Up (mo)
(45)	Uncontrolled trial	6	WG	CS, CTX, IFX	5	6 to 24
(46)	Case study	1	WG	CS, CTX, IFX	1	7
(47)	Uncontrolled trial	32	WG/MPA	CS, S/T, CTX, AZ, MTX, MMF, IFX	28	Maximum 12
(54)	Uncontrolled trial	10	WG (7)/other (3)	CS, IFX[b]	CR 5; PR 5	6
(55)	Case report	1	Pauci-immune NCGN	CS, S/T, IFX	1	12
(56)	Controlled trial	181	WG	CS, CTX or MTX, AZ, S/T, ETA or placebo	Experimental 69.7%; control 75.3%	>9 (mean 27)

[a]AZ, azathioprine; CR, complete remission; CS, corticosteroids; CTX, cyclophosphamide; ETA, etanercept; IFX, infliximab; MMF, mycophenolate mofetil; MPA, microscopic polyangiitis; MTX, methotrexate; PR, partial remission; S/T, sulfamethoxazole/trimethoprim; WG, Wegener's granulomatosis.

[b]Two patients with severe disease received additional therapy with CTX and with MTX and MMF, respectively.

achieved in a patient with microscopic polyangiitis after primary treatment with prednisolone, cotrimoxazole/trimethoprim, and four infusions of infliximab supports this notion (55).

In contrast to data on the use of infliximab, the effect of etanercept in patients with WG is less promising. In the only double-blind, placebo-controlled, multicenter trial on the use of TNF-α–inhibiting therapy in ANCA-associated vasculitis presented thus far, the Wegener's Granulomatosis Etanercept Trial (WGET) Research Group investigated the efficacy of the addition of etanercept to standard therapy (56). Patients with limited and severe disease, either newly diagnosed or relapsing, were enrolled. Standard treatment for limited and severe disease consisted of glucocorticoids and of methotrexate or cyclophosphamide, respectively; etanercept or placebo was randomly added to this as experimental treatment. Between the etanercept and placebo groups, no significant differences were found in remission rates; disease flares; and disease activity, damage, or quality-of-life scores. Remarkably, during follow-up, six cases of cancer were identified in the etanercept group, whereas no such events were seen in the control group. No significant differences were found in the incidence and the severity of other adverse events.

The results of the etanercept study are surprisingly negative and seem to be in remarkable contrast to the data that were obtained from animal studies and clinical trials using infliximab. A discrepancy between the effect of etanercept and infliximab was observed previously in another granulomatous disease, Crohn's disease (37), and potentially could be explained from differences in the working mechanism of the drugs. First, infliximab binds soluble and membrane-bound TNF-α much better than etanercept, resulting in an increased capacity of infliximab to inhibit TNF-α–mediated cytotoxicity and TNF-α–induced endothelial cell activation (57). Second, infliximab but not etanercept can induce an anti-inflammatory response by reverse signaling through membrane-bound TNF-α (58). Finally, infliximab and etanercept exert different effects on T lymphocytes (59,60) and monocytes (61).

Limitations to TNF-α–Inhibiting Therapy

From the studies on TNF-α inhibition in ANCA-associated vasculitis, as well as from similar studies that were performed in patients with other autoimmune diseases, several lessons can be drawn with respect to safety issues regarding this therapy. Generally, TNF-α–inhibiting therapy can lead to infusion reactions, such as headache, irritation at the site of injection, dizziness, nausea, chest pain, dyspnea, and pruritus. In addition, treatment with infliximab is associated with a higher rate of infections (6,49), in particular with an increased risk for reactivation of tuberculosis (62). In line with these findings, treatment with infliximab in patients with ANCA-associated vasculitis resulted in infections in 22% of the patients (47). However, in patients who had WG and were treated with etanercept (56), as well as in several controlled trials on infliximab in rheumatoid arthritis (5) and ankylosing spondylitis (8), the incidence of infection was similar in the treatment and control groups.

The role of TNF-α in carcinogenesis is complex and incompletely understood (63). The effect of anti–TNF-α therapy on the development of malignancies has been studied predominantly in rheumatoid arthritis, a disease that is associated with a higher incidence of malignancies (64). In this disease, a large case series (65), a controlled retrospective study (66), a large prospective study (64), and a controlled trial (6) suggest that treatment with infliximab or etanercept makes patients more prone to the development or recurrence of cancer, in particular lymphomas. Conversely, Lipsky *et al.* (5) found in a large prospective study that the incidence of malignancies in patients who had arthritis and were treated with infliximab was similar to background levels. In the WGET, the treatment of WG with etanercept increased the incidence of solid cancer (56), whereas one of 32 patients with ANCA-associated vasculitis was reported to have developed a B cell lymphoma during treatment with infliximab (47). Therefore, the link between TNF-α inhibition and malignancies should be investigated in more detail before any conclusion can be drawn with respect to this issue.

The high incidence of venous thromboembolic complications entails a new potential threat for patients with Wegener's disease (67). Bearing this in mind, it is of particular concern to gather information on the thrombogenic effects of TNF-α–inhibiting therapy. Whereas TNF-α generally is thought to be prothrombotic *in vivo* (reviewed in reference [68]), the putative anticoagulant effect of TNF-α inhibition is challenged by the finding that anti–TNF-α can have a prothrombotic effect in chimpanzees (69). In addition, it has been shown that treatment with infliximab or etanercept can induce the formation of potentially prothrombotic anticardiolipin autoantibodies (70). In the WGET, however, no prothrombotic effect of etanercept was found (56).

Treatment with infliximab as well as etanercept has been associated with increased levels of autoantibodies and/or a lupus-like autoimmune disease (reviewed in reference [71]). This has been studied most extensively in patients with rheumatoid arthritis (5,6,70,72–75), but anti–TNF-α treatment also was shown to induce autoimmunity in ankylosing spondylitis (75), Crohn's disease (51,52), and mixed connective tissue disease (76). The development of autoantibodies in these patients only very rarely leads to a (usually mild) lupus-like syndrome that resolves after discontinuation of anti–TNF-α therapy. However, anti–TNF-α therapy was complicated by the development of more severe glomerulonephritis in five patients who had rheumatoid arthritis and were treated with etanercept, infliximab, or adalimumab (77). In the WGET (56), as well as in the infliximab study (47), the incidence of autoimmunity was not reported. Nevertheless, autoimmunity that is induced by anti–TNF-α therapy remains an issue of concern in patients with ANCA-associated vasculitis as well.

Conclusion

Taken together, *in vitro* data support the notion that TNF-α plays an important role in the pathogenesis of ANCA-associated vasculitis and NCGN. Consequently, TNF-α bioactivity-inhibiting therapy might be effective in this disease. This is supported further by several studies in animal models of crescentic GN, although caution should be taken when translating

animal studies to the clinical situation. In the only controlled study on TNF-α bioinhibition in patients with ANCA-associated vasculitis, addition of etanercept to standard immunosuppressive therapy did not lead to improvement and was associated with the occurrence of serious adverse effects. It remains to be seen whether other approaches will be effective. In future studies, patients whose disease is refractory to current treatment strategies should be tested in a controlled manner, and infliximab should be tested instead of etanercept as additional therapy to standard immunosuppressive regimens. Thus far, only 65 patients with ANCA-associated renal disease have been reported to be treated with anti–TNF-α therapy. Lack of control subjects, publication bias, and the short-term nature of the reports severely limit any conclusions regarding efficacy. Therefore, patients should receive anti–TNF-α treatment only in a randomized, controlled manner or in the case of life-threatening disease, when no alternative therapeutic options are available. The incidence of potential adverse effects, such as infections, malignancies, thromboembolic complications, and autoimmunity, should be of specific concern.

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