Mechanisms of Differential Immunogenicity of Tumor Necrosis Factor Inhibitors

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Introduction

Advances in the understanding of the pathogenesis of rheumatoid arthritis (RA) have resulted in dramatic changes in the recommended treatment for patients with RA over the past decade. The goal of optimal management has gone from reducing the probability of irreversible joint damage [1] to preventing joint damage [2]. Patients were once moved through a pharmacologic "pyramid" based upon minimization of toxicity as a primary consideration: nonsteroidal anti-inflammatory drugs (NSAIDs) at its base, single disease-modifying antirheumatic drugs (DMARDs), and combination DMARDs at its apex [1,3]. With the recognition that irreversible joint damage often occurs within the first 2 years of disease, the treatment pyramid has been turned upside down with more aggressive early treatment of the disease. Today, initiation of DMARD therapy is recommended for the majority of patients within 3 months of diagnosis of RA [2].

Tumor necrosis factor (TNF) antagonists represent a further major advance in the treatment of RA. TNFα is an essential mediator in the cytokine inflammatory cascade in RA. In animal models of arthritis, TNF blockers (*eg*, neutralizing antibodies, receptor antagonists) prevented arthritis, suggesting that overexpression of TNF is necessary and sufficient to produce joint inflammation [4–6]. These proof-of-principle experiments provided the foundation for clinical trials showing that TNF blockade can relieve joint inflammation and potentially inhibit progression of disease in patients with RA [7–11]. Moreover, TNF antagonists target a specific aspect of the immune response, as opposed to other more generally immunosuppressive DMARDs.

Drugs currently marketed in the United States include the monoclonal antibodies, infliximab (Remicade; Centocor, Malvern, PA) and adalimumab (Humira; Abbott Laboratories, Abbott Park, IL); and a fully human soluble TNF receptor, etanercept (Enbrel; Immunex Corporation, Thousand Oaks, CA). All three drugs selectively target TNF, but they differ in their pharmacodynamics, pharmacokinetics, immunogenicity, and possibly mechanism of action. Pharmacodynamic and pharmacokinetic differences have been reviewed elsewhere [12–14]. This paper focuses on the extent that host immune response differs among patients treated with monoclonal antibodies to TNF as compared with those treated with the soluble TNF receptor. We also explore factors that complicate attempts to compare immunogenicity among different biologics and suggest ways that future comparisons could be made more useful for clinicians.

Molecular Structure and Mechanisms: Relationship to Immunogenicity

All three biological agents improve clinical symptoms and signs of RA [15]. The mechanism of this improvement differs between monoclonal antibodies and the soluble receptor.

The human soluble receptor product, etanercept, is a fusion protein that combines soluble TNF type-2 receptor with the constant region (Fc) of human immunoglobulin (IgG1) (Fig. 1). This fusion protein binds to circulating and membrane-bound TNF to reduce the amount of inflammatory cytokine available for receptor binding [12].

The monoclonal antibodies consist of two variable regions that contain the antigen-binding fragments (Fab region) of human or mouse immunoglobulin, combined with the Fc region of human IgG1 (Fig. 1). The Fab region binds to soluble TNFα as well as to cell-surface-bound TNFα. Infliximab forms more stable complexes with transmembrane TNF than does etanercept [16]. When the monoclonal antibodies bind to cell-surface TNF, the Fc portion of the IgG1 region of the molecule can activate complement-dependent cytolysis and antibody-dependent cell-mediated cytotoxicity [17]. This direct killing of cells expressing membrane-associated TNF, such as macrophages and monocytes, may explain the monocytopenia observed in patients after being treated with infliximab that can persist for weeks after infusion [18]. Given the identical constant regions of both

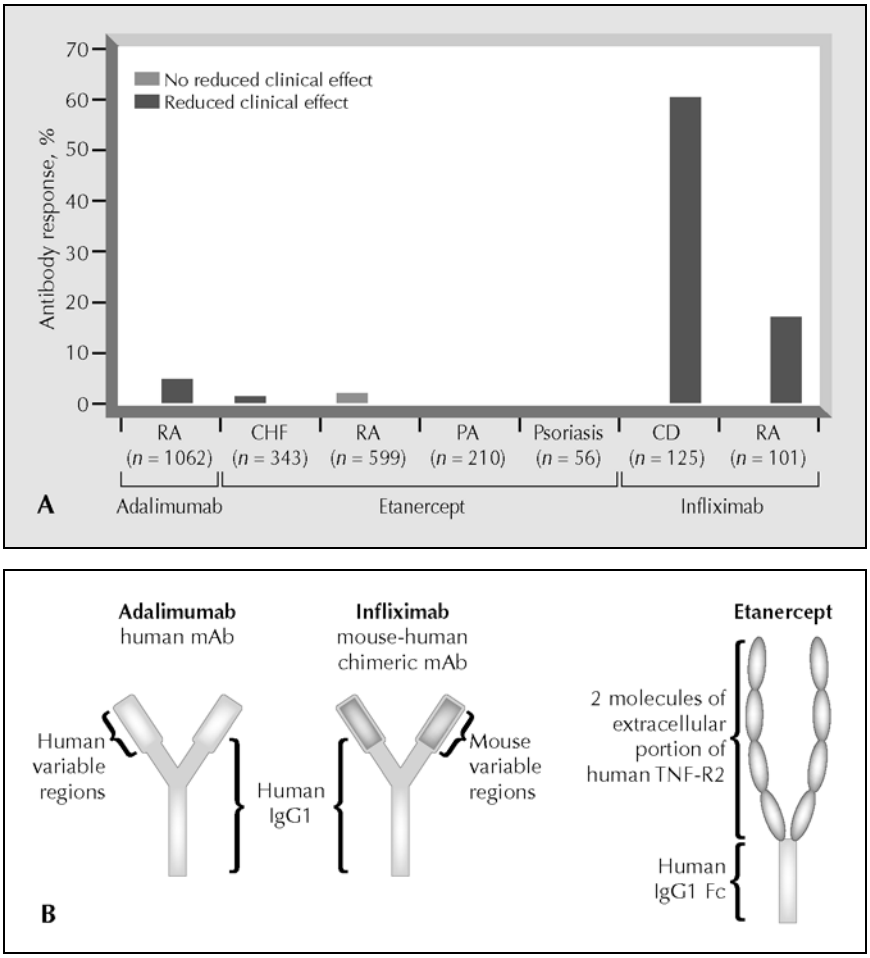

Figure 1. **A**, Summary of antibody response to tumor necrosis factor inhibitors. **B**, Molecular structure of monoclonal antibodies (adalimumab and infliximab) and soluble receptor protein. Ab—antibody; CD—Crohn's disease; CHF—congestive heart failure; IgG—immunoglobulin G; PA—psoriatic arthritis; RA—rheumatoid arthritis; TNF—tumor necrosis factor.

molecules, adalimumab may have a similar effect. While etanercept contains the Fc portion of IgG1, unlike the monoclonal antibodies etanercept only produces monomeric or dimeric complexes with TNF, and may be insufficient to activate complement.

Studies also suggest that infliximab may affect activated monocytes and T lymphocytes. In Crohn's disease (CD), infliximab may exert its sustained therapeutic effects by causing apoptosis of T lymphocytes [19–20]. Apoptosis has also been observed in circulating monocytes from CD patients after infliximab infusion [21]. There is no evidence that etanercept exerts its therapeutic effect through apoptosis.

Host Immune Responses

Although infliximab and adalimumab are monoclonal antibodies, the two differ in the origin of their component parts (Fig. 1). Infliximab is a mouse/human chimeric monoclonal antibody that contains two mouse variable regions joined to a human constant region [22]. Adalimumab is a human monoclonal antibody made through recombinant technology using human B cell lines [23].

Mouse proteins are immunogenic; that is, they are recognized by the human immune system as foreign and elicit an immune response. The resultant host antibodies are called human antimurine antibodies (HAMA; also known as human antichimeric antibodies or HACA).

Human antimurine antibodies can negatively affect the function of monoclonal antibodies. "Neutralizing" HAMA attach to the TNF-binding sites of the monoclonal antibodies and prevent interactions with native TNF; thus blocked, the drug molecule can no longer exert its intended function. Non-neutralizing HAMA bind to regions other than the TNF-binding region and therefore do not prevent the drug's binding to TNF, but these host antibodies may promote the clearance of the drug by promoting complement binding and clearance by macrophages. It is also possible that non-neutralizing HAMA can affect the drug's conformation in a way that reduces its efficacy.

Even human monoclonal antibodies can trigger an immune response. Despite being completely of human origin, the unique antigen-binding site on these antibodies is capable of triggering an immune response. The TNF-binding region of adalimumab, for example, despite being derived from human amino acid sequences, is not an antibody region that would be typically expressed in all adults. The production of human antihuman antibodies (HAHA) results when the host immune system recognizes this

unique TNF-binding region as "nonself" and develops antibodies against it. HAHA production is less common than the production of HAMA.

Soluble receptor fusion proteins like etanercept tend to induce a weaker immune response, if one develops, than do monoclonal antibodies. In contrast to the TNF-binding domain on the monoclonals, the TNF-binding domain on the soluble receptor is the naturally occurring human TNF receptor and is therefore not recognized as "nonself." However, the junction between the TNF receptor and the immunoglobulin domain may take on a unique conformation that can be recognized by the immune system. That is, the "hinge" region, where a TNF receptor arm meets the IgG1 Fc portion, does not occur naturally. Consequently, host antibodies reactive with etanercept are observed in some patients. As they are directed at the "hinge" region, these antibodies are non-neutralizing and do not interfere with TNF binding [24].

Immunogenic Responses to Tumor Necrosis Factor Inhibitors

A number of clinical studies have evaluated the immunogenicity of TNF inhibitors. However, a lack of standardization and validation of the enzyme immunoassays commonly used to quantitatively assess host antibodies, as well as differences in underlying populations tested, make it difficult to directly compare immunogenic response among the different anti-TNF agents. One critical issue, for example, is the specific technique used for the enzyme-linked immunosorbant assay (ELISA) used for antigen measurements. There are two available techniques that follow basic ELISA principles: the double-antigen technique and the competitive technique. Only the competitive technique can identify whether antibodies are neutralizing (bind to the TNF receptor site of the drug molecule and prevent TNF binding) or non-neutralizing (bind to another site on the drug molecule). The double-antigen assay, also known as the sandwich or bridging technique, detects the presence of antibodies, but does not specify the type (neutralizing vs non-neutralizing). The nature of the ELISA also makes it prone to high interassay variability and false-positive and negative signals. Rheumatoid factor, which may bind the Fc fragment of IgG, may cause positive interference in the assays. Slight changes in the plating concentration of the antibody, differing cutoff calculations, and varied reagents can significantly change the results of a test. Furthermore, there are high rates of doubtful and weakly positive test results. Despite its pitfalls; however, ELISA tests correlate well with clinically observed findings (such as the occurrence of severe infusion reaction in patients with high antibody titers) [25] and have been used extensively in academic and clinical research.

Human antimurine antibodies

One large multicenter trial in infliximab-treated RA patients measured the presence of HAMA using a modified ELISA based on the double-antigen technique [8]. Samples were collected 12 weeks after the last infusion of infliximab to allow for clearance of the drug. Those samples with infliximab still present were considered to have a negative HAMA response. The overall incidence of HAMA in all infliximab-treated patients (n = 101) was 17.4%. The occurrence of HAMA was inversely proportional to the infliximab dosage. Concomitant therapy with methotrexate diminished the appearance of HAMA (Table 1). Compared with a lower dose, a higher dose of infliximab elicited greater responses to treatment, as measured by the Paulus 20% index, and a lower occurrence of immunogenic response. Concomitant treatment with methotrexate at the 1 mg/kg dosage of infliximab conferred a longer response to treatment compared with infliximab treatment alone and placebo (Table 1).

This study suggests that high-dose infliximab treatment promotes immunologic tolerance to infliximab. The difference in immunogenicity between the high and low doses may lie in the maintenance of circulating levels of infliximab with the higher doses. This phenomenon of high zone tolerance may occur when high levels of infliximab produce tolerance in B cells in the bone marrow and prevent them from subsequently mounting an antibody response [26].

Methotrexate, when used with higher doses of infliximab, drastically mitigates the immunologic response. Baert *et al*. [25] studied the influence of immunogenicity on long-term efficacy of infliximab in CD patients by measuring the concentrations of infliximab and anti-infliximab antibodies in 125 consecutive infliximab-treated CD patients. The immunogenicity of infliximab was assessed using an assay based on the double-antigen technique of ELISA. Antibodies against infliximab were detected in 61% of patients. The presence of anti-infliximab antibodies greater than or equal to 8.0 μg/mL before an infusion was associated with a shorter duration of response and a higher risk of infusion reactions (Table 1) (relative risk, 2.40; 95% confidence interval, 1.65–3.66; $P <$ 0.001). The cumulative incidence of infusion reactions was 27%. Patients who experienced infusion reactions had significantly lower infliximab concentrations at 4 weeks (median, 1.2 vs 14.1 μg/mL; $P < 0.001$) and a shorter duration of clinical response (median, 38.5 vs 65 days; $P < 0.001$) compared with patients who never had an infusion reaction. Concomitant immunosuppressive therapy was predictive of low titers of anti-infliximab antibodies ($P < 0.001$) and high infliximab concentrations 4 weeks postinfusion ($P < 0.001$).

This study indicates that the development of antibodies against infliximab is associated with an increased risk for infusion reactions and a reduced duration of response to treatment due to lower infliximab concentrations. In addition, the need for increasing doses of infliximab over time has been observed in non-clinical trial settings. In a prospective, multicenter study, 57% of 135 RA patients receiving infliximab required an increased infliximab dosage or shortened dosing interval to maintain symptomatic control over a 20-month period [27]. A retrospective observational

Table 1. Clinical immunogenicity of tumor necrosis factor

Study	Drug/population	Antibody response, %	Clinical outcomes
Maini *et al.* [8]	Infliximab; RA (*n* = 101)	HAMA Overall, 17 1 mg/kg, 53 3 mg/kg, 21 10 mg/kg, 7 w/MTX 7.5 mg/week 1 mg/kg, 15 3 mg/kg, 7 10 mg/kg, 0	Higher doses elicited lower immunogenic response and greater response to treatment (Paulus 20% index)
Baert *et al.* [25]	Infliximab; CD (*n* = 125)	Overall (by 5th infusion), 61 ≥ 8 μg/mL, 37 + Immuno, 43 No immuno ($P < 0.01$), 75	Duration of response, ≥ 8 μg/mL Ab: 35 days; < 8 μg/mL Ab: 71 days; ($P < 0.001$); Cumulative incidence infusion reactions: 27%
Kress [29]	Adalimumab; RA (*n* = 1062)	HAHA 20 mg/mL Biweekly dosing 17.9 Weekly dosing, 9.8 40 mg/mL Biweekly dosing, 17.7 Weekly dosing, 3.9 MTX, 1 No MTX, 12	ACR 20 response (40 mg adalimumab), Ab (+) 30%; Ab (-) 50%
Foerder and Rogge [30]	Etanercept; RA (*n* = 599); CHF (*n* = 343); Psoriatic arthritis (*n* = 210); Psoriasis (*n* = 56)	Overall, 1.2 RA, 2.0 CHF, 0.6 Psoriatic arthritis, 0 Psoriasis, 0	

Ab—antibody; ACR—American College of Rheumatology; CD—Crohn's disease; CHF—congestive heart failure; HAHA—human antihuman antibodies; HAMA—human antimurine antibodies; Immuno—immunosuppresive agent; MTX—methotrexate; RA—rheumatoid arthritis.

study using medical claims also reported significant increases in average infliximab dosages in the year after infliximab initiation [28]. Concomitant immunosuppressive therapy reduces the magnitude of the immunogenic response by preventing infusion reactions and maintaining clinical efficacy.

Human antihuman antibodies

Kress [29] reviewed one Phase 2 and two Phase 3 multicenter, placebo-controlled clinical trials of adalimumab in RA. A total of 1062 patients received adalimumab and were tested at multiple time points for antibodies to adalimumab using the double-antigen format of ELISA. Low-titer neutralizing HAHA at titers greater than 20 ng/mL developed in 6% of adalimumab-treated patients and less than 1% of placebo-treated patients at least once during treatment. HAHA occurred more frequently in patients who received biweekly doses of adalimumab than in those who received weekly doses (Table 1). Among patients treated biweekly, HAHA occurred more frequently in those who received 20 mg adalimumab than in those who received 40 mg (Table 1). Furthermore, patients treated with concomitant methotrexate had a lower rate of HAHA development than did patients on adalimumab monotherapy. The presence of HAHA impacted efficacy: at 40 mg, the American College of Rheumatology 20 response was lower among antibody-positive patients than among antibody-negative patients. The presence of HAHA was not associated with clinically meaningful differences in the incidence of treatment-emergent adverse events or withdrawals. The long-term impact of immunogenicity on the need for dose escalation of adalimumab is unknown.

Antibodies against etanercept

Foerder and Rogge [30] reported observations of the development of immune responses to etanercept therapy in RA, psoriatic arthritis, psoriasis, and congestive heart failure (CHF) patients who participated in controlled clinical trials. The authors compared specific immune responses with etanercept by using a competitive ELISA format designed to optimize the specificity and sensitivity of the assay, including using different coating concentrations in the assay and minimizing the positive interference of rheumatoid factor. In a test population of a subset (n = 750) of the RA patients, the incidence of antibody-positive samples ranged from less than 1% to 18% depending on the assay configuration, indicating that minor differences in assay construction or cutoff calculation can result in significantly different conclusions about relative immunogenicity. For the subsequent analyses, the assay configuration determined as most appropriate by the study sponsor and the U.S. Food and Drug Administration was used [31].

The overall prevalence of immunogenicity of etanercept in these studies was 1.2% (14/1208). Two percent of etanercept-treated RA patients in five different clinical trials were antibody positive, while 0.6% of etanercept-treated CHF patients from two trials were antibody positive. None of the psoriatic arthritis patients or psoriasis patients who received etanercept in clinical trials was antibody positive (Table 1). All antibody-positive specimens were subsequently demonstrated to be non-neutralizing. No correlation was observed between a positive result in the anti-etanercept assay and any impairment of the therapeutic effects of the drug, a distinct difference from the monoclonal antibodies in which antibody presence was associated with lower efficacy [8,29].

While reported estimates of relative immunogenicity of etanercept may vary somewhat based on the assay used, clinical findings consistently indicate that etanercept has very low incidence of immunogenicity in the populations studied, and that the anti-etanercept antibodies are non-neutralizing and do not affect its therapeutic effects and safety.

Conclusions

The immunogenicity of TNF inhibitors is an important factor that may affect the tolerability and long-term efficacy of these agents. Research suggests that the anti-TNF monoclonal antibodies infliximab and adalimumab are immunogenic, but etanercept, the soluble receptor fusion protein, may be less so (Figure 1). Antibodies to the monoclonals may lower efficacy and increase the risk for infusion reactions. Antibodies to the soluble receptor do not appear to have these effects. Moreover, infliximab, as a chimeric antibody, appears to induce a greater immunogenic response than does adalimumab, the human monoclonal antibody.

While it would be desirable to compare the relative immunogenicity of TNF agents through published clinical studies, interpretation of ELISA results is challenging. Researchers develop their own customized assays and there is no standardized test of sensitivity or specificity. Variation in the use of washout periods to eliminate circulating drug also affects test results. In the studies reviewed, many test results were deemed "indeterminate" for the presence of antibodies because of the detection of drug in the assay. The difficulty in assessing immunogenicity of anti-TNF agents based upon ELISA testing is highlighted in two comparisons. First, in the two studies evaluating immunogenic responses in patients treated with infliximab, the overall immunogenic response was very different (17.4% vs 61%). This dramatic difference may reflect differences between the underlying populations: antibodies to infliximab may be strikingly more common in CD as compared with RA patients. However, the findings of Foerder and Rogge argue against this being the only cause of this wide variation in immunogenic response. They used different permutations of the ELISA on an identical population (*ie*, within the RA population). Varying their ELISA protocol changed the incidence of antibody detected from less than 1% to 18%, suggesting that within-population variation may be just as large as across-population variation.

Ultimately clinical outcomes trump findings in an ELISA assay. That is, if one agent has demonstrably better clinical outcomes, then laboratory findings will be secondary. Clinical studies support the use of the monoclonal anti-TNF antibodies and the soluble TNF receptor fusion proteins in the treatment of RA and other inflammatory diseases. Although the use of combination therapy with methotrexate generally reduced the immunogenicity of the monoclonal antibodies, the relatively greater immunogenicity of the monoclonal antibodies cannot be ignored. The development of these antibodies, particularly those of the neutralizing type, may explain the need for dose escalation seen in clinical practice with monoclonals

Immunogenicity of a drug therapy has important implications for effective treatment of a disease. However, the immunogenicity of a specific therapy is not easy to discern. As biologic therapies become more common, there will be an even greater need for standardization of assays that measure immunogenicity. If drug manufacturers and researchers are not able to agree on standard methods for testing, regulatory intervention may be needed to give clinicians adequate information on which to base treatment decisions.

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