Review Article

Impact of TNF inhibitors on rheumatoid arthritis

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Tumor Necrosis Factors (TNFs) have an important role in host defense against infections, while accumulating evidence suggest that overproduction of TNFs is involved in the pathogenesis of inflammatory disorders such as Rheumatoid Arthritis (RA). Early experiments have demonstrated that RA synovial cells produced an excess amount of TNF- α *in vitro*, and an anti-TNF- α monoclonal antibody inhibited not only TNF- α , but also subsequent production of IL-1, IL-6, and IL-8, suggesting that TNF- α is located upstream of the cytokine cascade. In addition, TNF- α transgenic (tg) mice developed erosive polyarticular arthritis, and the blockade of TNF- α significantly reduced the severity and the development of arthritis, and joint destruction. These results raise a possibility that the TNF inhibition is useful to manage inflammatory arthritis and has facilitated the clinical development of this approach. In this review, we introduce the physiological and pathological roles of TNFs, and summarize the efficacy and safety of the biological agents targeting on the tumor necrosis factor. The biological TNF inhibitors provide a great impact on our understanding of the pathogenesis, and have revolutionized our strategy in the management of inflammatory disorders with unknown etiology.

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Introduction

Tumor necrosis factor (TNF) can kill tumor cell lines *in vitro*¹. Two other factors with a similar *in vitro* activity have also been reported and have been designated as lymphotoxin and (LT- α , LT- β)²⁻⁴. TNFs clearly have an important role in host defense against infections, but their potential role in tumor development and progression in humans remains uncertain^{4,5}. On the other hand, accumulating evidence has suggested that the overproduction of TNFs is involved in the pathogenesis of inflammatory disorders like rheumatoid arthritis (RA) and other inflammatory conditions^{6,7}. A substantial effort has been made to develop biological agents that specifically inhibit the function of TNFs. A chimeric monoclonal antibody against TNF- α was

generated and its efficacy and safety tested in a limited number of RA patients in the early 90s⁸. The efficacy was surprisingly excellent, and the antibody was well tolerated. These results were then corroborated in a number of randomized controlled studies not only for RA, but also for other inflammatory disorders like Crohn's disease, ankylosing spondylitis, and psoriatic arthritis^{9,10}. Such biological TNF inhibitors have improved our understanding of the pathogenesis of inflammatory disorders and have revolutionized our strategy for the management of inflammatory disorders of unknown etiology^{11,12}.

Structure and function of TNF

Within the family of TNFs, TNF- α , TNF- β (also known as

Fig.1 Structures of TNF family members and receptors

lymphotoxin α , lymphotoxin β , and several other lymphotoxins have been identified so far. These first three molecules are generated from three distinct genes on the third chromosome and function in the form of trimers that bind to receptors consisting of homotrimers⁵. TNF α and LT α (TNF β) can bind to two kinds of TNF receptors, TNF receptor I (p55) and TNF receptor II (p75), which are ubiquitously expressed on cell surfaces throughout the body (Fig.1)^{4,5}. A unique heterotrimer, consisting of one molecule of LT α and two molecules of LT β , binds to a third receptor, designated as LT β R. Each of the receptor molecules has long been thought to exist independently and to assemble upon ligand binding. This model is known as the ligand-induced trimerization model. Since signal transduction delivers distinct signals, depending on the individual receptor class¹³, it is widely accepted that a single type of receptor can come preassembled as a trimer. This pre-ligand assembly model has now been confirmed, since a pre-ligand assembly domain (PLAD) has been identified¹⁴. Recently, biologically engineered PLADs have been shown to ameliorate arthritis in animal models, supporting the pre-ligand assembly hypothesis¹⁵.

TNF α is produced by a variety of cells, including monocytes/macrophages, T cells, B cells, and keratinocytes⁵. On the other hand, LT α is mainly produced from naive T cell populations¹⁶. Upon stimulation, signals are transduced from cell surface receptors into the cytoplasm¹³, ultimately causing the transcription factors to bind to the promoter region of the targeted genes and the initiation of TNF α transcription. The stability of TNF α mRNA is regulated by stabilizing and destabilizing factors that bind to an AU-rich region located within the 3' untranslated region (UTR) of the mRNA. One of these stabilizing factors is HuR, while TIA-1 acts as a destabilizing factor. TNF α thus produced with the transmembrane domain is transported to the surface of the cell membrane, where it is expressed.

Fig.2 Pathogenesis of RA

When membrane proteases like TNF α -converting enzyme (TACE) or membrane-type metalloproteinases (MT-MMP) cleave to TNF α on the cell surface under pathological or physiological conditions, respectively, free TNF α is released⁵.

Excessive amounts of TNF α in the serum alter the anticoagulant activity of endothelial cells, inducing hemorrhage, vasodilatation, and neutrophil activation - the features of septic shock. On the other hand, a small but sustained increase may evoke inflammatory responses, like fever, anemia, body weight loss, and even osteoclast activation⁶.

Role of TNF α in the pathogenesis of RA

Early experiments demonstrated that RA synovial cells produce an excess of TNF α *in vitro* and that an anti-TNF α monoclonal antibody inhibits not only TNF α , but also the subsequent production of IL-1, IL-6, and IL-8; these findings suggested that TNF α is located upstream of the cytokine cascade^{6,7}. Immunohistochemical staining of synovial tissues from RA patients showed the enhanced expression of TNF α not only in synovial lining cells, but also in sublining tissues; staining for LT α was less prominent¹⁷. Although LT α plays a crucial role in peripheral lymphoid organ development and partially contributes to sustaining inflammation¹⁸⁻²⁰, the above results suggest that TNF α is the major player in most RA patients. To test the hypothesis that TNF α plays a pivotal role in the pathogenesis of RA, TNF α transgenic (tg) mice were generated. The TNF α tg-mice developed erosive polyarticular arthritis, but TNF α blockade significantly reduced the severity and even the development of arthritis and joint destruction. Furthermore, an anti-TNF α monoclonal antibody ameliorated collagen-induced arthritis²¹. These results suggest that TNF inhibition may be useful for the management of inflammatory

Fig.3 Biological agents targeting TNF

arthritis and have facilitated the clinical development of such an approach¹¹⁾.

As shown in Fig.2, TNF- appears to play a central role in the pathogenesis of RA through the induction of adhesion molecules in endothelial cells, the production of vascular endothelial growth factor (VEGF), the proliferation of synovial cells, and, most importantly, the activation of osteoclasts.

Biological agents inhibiting the activity of TNF

Several strategies have been adopted to produce biological agents that can inhibit the biologic activity of TNF-. Two major categories of agents exist: monoclonal antibodies and receptor-antagonist/receptor-Fc fusion proteins²²⁾. As shown in Fig.3, several monoclonal antibodies including a chimeric monoclonal antibody to TNF- (clone cA2: infliximab, trade name: Remicade®⁸⁾), a humanized mAb (CDP571)²³⁾, human mAbs (clone D2E7: adalimumab, trade name: Humira®, clone 148: golimumab)²⁴⁾, and a polyethylene glycol (PEG)-Fab fragment (CDP870) have been generated. Several receptor antagonist/receptor-Fc fusion proteins like a TNF receptor II-Fc fusion protein (etanercept, trade name: Enbrel®²⁵⁾), a TNF receptor I-Fc fusion protein (Lenercept), and a PEG-soluble TNF receptor type I (Pegsunercept)²⁶⁾ have also been developed. Infliximab, adalimumab, and etanercept have been approved for medical use in the United States and are being used in clinical settings. The first biological agent approved in the United States for the treatment of RA was etanercept (1998), followed by infliximab (1999). In 2002, infliximab was introduced to the Japanese market for the treatment of Crohn's disease; its application was expanded to include the treatment of RA in 2003. Etanercept was approved in Japan for the treatment of RA in 2005.

Table 1 Characteristics of TNF inhibitors

TNF inhibitors	infliximab	etanercept	adalimumab
Structure	Chimeric mAb	Human IgG fusion	Fully human mAb
Half-life	8 - 10 days	4.2 days	10 - 14 days
C' binding	(+)	(-)	(+)
TNF+ cell lysis	(+)	(-)	(+)
Antibody development	10% (+MTX) Human anti-chimeric antibody: HACA	< 5% (non-neutralizing)	1 - 12% (-MTX) (5%: neutralizing) Human anti-human antibody: HAHA
MTX	With	With and without	With and without
Dose	3 mg/kg at 0, 2, 6 w and every 8 w (Japan)	25 mg 2/w SQ (Japan)	40 mg 1/1-2w SQ (USA)
Indications (worldwide)	RA, Crohn, PsA, AS, UC	RA, JIA, PsA, AS	RA

(JIA: Juvenile idiopathic arthritis, PsA: Psoriatic arthritis, AS: Ankylosing spondylitis, UC: Ulcerative colitis)

The administration of etanercept is indicated for the treatment of RA, JIA, psoriatic arthritis, and ankylosing spondylitis, whereas the administration of infliximab is indicated for the treatment of RA, Crohn's disease, psoriatic arthritis, ankylosing spondylitis, and ulcerative colitis (see Table 1). Humira is indicated for the treatment of RA.

Characteristics of individual TNF inhibitors

Infliximab is a chimeric mAb consisting of a variable region of mouse immunoglobulin (Ig) that binds to TNF- with high affinity ($K_a = 1.8 \times 10^9$) and the Fc portion of human IgG1 (Fig.3). The half-life of infliximab is 8-10 days. Infliximab fixes complements and induces the lysis of TNF-expressing cells, such as macrophages. An anti-chimeric antibody (HACA) can be detected in approximately 10% of all patients with MTX (Methotrexato) and in up to 40% of all patients without MTX (Table 1).

Etanercept is a genetically engineered protein consisting of two molecules of the extracellular domain of TNF receptor II (p75) and the Fc fragment of IgG1 (Fig.3). The half-life of etanercept is 4.2 days, and it does not fix complements or induce the lysis of TNF(+) cells. While antibodies against etanercept can be detected in less than 5% of all patients, a neutralizing antibody is not thought to be produced (Table 1).

Adalimumab is a full human monoclonal antibody of IgG1 that binds to TNF- with high affinity ($K_d = 6 \times 10^{-10}$ M). An antibody with the clone name of D2E7 is made by the phage display method using the amino acid sequence from the human germ line (Table 1). The half-life of adalimumab is 10-14 days, and it fixes complements and induced the lysis of TNF-expressing cells. An anti-human antibody (HAHA) can be detected in

Fig.4 Action of TNF inhibitors

1-12% of all patients, while a neutralizing antibody is observed in 5% of all patients (Table 1).

Fig.4 is a schematic representation of the action mechanisms of these TNF-inhibiting biological agents. Infliximab and adalimumab bind and neutralize the activity of serum TNF- α . These two monoclonal antibodies can also bind TNF- α on the cell surface, fixing complements and inducing the lysis of these cells, thereby inhibiting TNF- α production. In contrast, etanercept binds not only TNF- α , but also TNF- α (LT- α), neutralizing these two cytokines; however, etanercept does not inhibit the production of these factors.

Efficacy of individual TNF inhibitors

Infliximab: Infliximab was first tested in patients with severe RA who had not responded to conventional DMARDs. A single infusion of 5-20 mg/kg clearly produced a striking reduction in the signs and symptoms of RA in these patients⁹. However, the serum concentration of infliximab gradually decreased, disappearing 3-4 weeks post-infusion, and the disease activity returned to its pre-treatment level, suggesting that repeated infusions are required to control disease activity for longer periods. To this end, clinical trials using repeated infusions of infliximab in RA patients were performed, and these trials demonstrated that the interval between infusions was reduced the infusions were repeated more than several times²⁷. Later, it has been demonstrated that low doses of infliximab such as 1 or 3 mg/kg in combination with MTX exhibited a clinical response comparable to that by high doses of infliximab (10 mg/kg) alone, breaking through these difficulties²⁸. Since the development of the human antibody to chimeric monoclonal antibody (HACA) was interfering with the infliximab activity²⁹, the dose sparing effect by MTX could be attributed to immunosuppression of HALA production.

Fig.5 Japanese clinical trials for infliximab

1) ATTRACT study: A comparison of MTX alone and MTX + infliximab

To confirm the efficacy of infliximab and MTX combination therapy, the Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis Concomitant Therapy (ATTRACT) study was conducted in 428 patients with severe RA who did not respond to MTX at a dose of more than 12.5 mg/week³⁰. After 54 weeks, the patients treated with MTX + a placebo infusion showed only a 17% response, whereas all of the infliximab-treated group showed a significant response, with 56% of the patients achieving ACR20%³¹. Surprisingly, the mean change in the modified sharp score, which represents a semi-quantitative measure of bone and cartilage destruction, was more than 7 in the placebo group (only MTX), whereas it was markedly suppressed to less than 1 in the infliximab-treated group³¹. These results suggested that infliximab in combination with MTX not only reduces the signs and symptoms of RA, but also strongly inhibits the radiographic progression of structural damage.

2) Japanese trial (Fig.5)

In Japan, a similar clinical trial was performed using three groups, with MTX + placebo, MTX + infliximab (3 mg/kg), or MTX + infliximab (10 mg/kg) administered at 0, 2, and 6 weeks; a significantly better clinical response was obtained in the infliximab-treated groups³². The percentage of patients treated with infliximab (3 mg/kg) who responded (according to the ACR20% criteria) at 14 weeks was 61.2%, while that in the placebo group was 23.4%. When the data from the Japanese trial and the ATTRACT study were compared, the Japanese trial ex-

hibited a better result at week 14 (37.8% for the Japanese trial, 21% for the ATTRACT study: ACR 20% [$\{ \text{ACR20\% in MTX} + \text{infliximab group} \} - \{ \text{ACR20\% in MTX} + \text{placebo group} \} \}$). One of the major distinctions in RA treatment between Western countries and Japan is the maximum MTX dosage, which is 8 mg/week in Japan, raising the possibility that Japanese RA patients receiving a lower maximum dose of MTX may exhibit milder disease activity than their Western counterparts and thus may respond better to infliximab. Other possibilities include differences in the genetic background associated with disease severity and differences in background characteristics like age or the duration of illness.

3) ASPIRE study: A comparison of MTX + placebo, and MTX + infliximab in patients with early RA

The effects of MTX alone or in combination with two possible doses of infliximab (3 mg/kg or 6 mg/kg) were compared in patients with early active RA (less than 3 years after the onset of RA) with poor prognostic factors. After 54 weeks, the combination of MTX and infliximab at either dose exhibited significantly high ACR20%, ACR50%, and ACR70% responses as well as a significantly smaller increase in the total sharp score, indicating that the effect of the combination therapy on early active RA was superior to that of MTX alone³³. The numbers of total and severe adverse events were comparable among the three treatment groups, supporting the idea that combination therapy should be considered in patients with early active RA who have a poor prognosis and in whom radiographic structural damage is progressing rapidly, leading to profoundly irreversible disability.

Etanercept: In early studies, the effects of a placebo were compared with those of etanercept (0.25 mg/m², 2 mg/m², or 16 mg/m²) in 180 RA patients who had not responded to treatment with between one and four previous DMARDs. After 3 months of treatment, a significant reduction in disease activity was observed in the etanercept-treated group, and a dose-related therapeutic effect was confirmed. In RA patients treated with 16 mg/m² of etanercept, 75% of the patients achieved an ACR20% response, whereas the placebo group only achieved a response of 14%²⁵. Thereafter, a randomized placebo-controlled trial using fixed etanercept doses, such as 10 mg and 25 mg twice a week for 24 weeks, was designed and carried out in 234 patients with active RA for 6 months; a profound reduction in all core set variables was obtained in the etanercept group, and the ACR20% response was 59% in the etanercept-treated group (25 mg, twice a week) but only 11% in the placebo group³⁴. In addition, a study was performed to examine the efficacy of etanercept in patients with inadequate responses to a stable dose of MTX. In

the group treated with etanercept (25 mg, twice a week), the ACR20% response rate was 71%, while the placebo group had a response rate of 27%, indicating that etanercept also exhibited a significant benefit in RA patients with inadequate responses to MTX³⁵. Follow-up studies showed a sustained clinical response for up to three years³⁶.

1) ERA study: A comparison of MTX and etanercept in patients with early RA

To demonstrate the efficacy of etanercept in patients with early RA, a direct comparison between etanercept and MTX was carried out in 632 patients who had been diagnosed as having RA for no more than 3 years and had not been treated with MTX³⁷. The patients were divided into three groups that received MTX (n=217), 10 mg of etanercept (n=208), or 25mg of etanercept (n=207). MTX was started at a weekly dose of 7.5 mg, followed by an increase of up to 15 mg/week at week 4 and 20 mg/week at week 8 with folic acid supplementation (1 mg/day). After the second dose escalation, the mean MTX dose was 19 mg/week, because 15% of the patients required a dose reduction because of adverse events. After 24 weeks, the patients who had been treated with 25 mg of etanercept showed a more rapid improvement, with higher ACR20%, ACR50%, and ACR70% response rates than the MTX-treated patients³⁷. A radiographic evaluation revealed that the mean increase in the erosion score was 0.30 in patients treated with 25 mg of etanercept and 0.68 in the MTX group, whereas joint-space-narrowing was comparable between the two groups. A two-year follow-up study demonstrated that the mean change in the total sharp score in the 25 mg of etanercept group was significantly lower than that in the MTX group (1.3 and 3.2, respectively), suggesting that etanercept acted more rapidly to reduce the clinical parameters and retard joint damage in early active RA³⁸.

2) TEMPO study : A comparison of MTX, etanercept, and MTX + etanercept in patients with early RA

Among 682 patients with early active RA (less than 3 years), patients treated with MTX + etanercept showed a significantly high ACR70% response and a high clinical remission rate according to the DAS (Disease activity score) definition, in addition to a significantly low mean sharp score below zero, compared with the patients treated with MTX or etanercept alone. These remarkable results indicate that combination treatment can realize remission in a much higher proportion of patients with early active RA and a poor prognostic profile than MTX alone or even etanercept alone; furthermore, the combined treatment may arrest or even repair structural damage³⁹.

Adalimumab: Two hundred eighty-four patients with active RA who had not responded to DMARDs were randomly assigned

to receive weekly subcutaneous injections of adalimumab (20 mg, 40 mg, or 80 mg) or a placebo without the concomitant administration of DMARDs for 12 weeks. Adalimumab significantly improved the signs and symptoms of RA, yielding significantly better ACR20% response rates (50.7%, 57.1%, and 54.2% for doses of 20 mg, 40 mg, and 80 mg, respectively, versus 10.0% for the placebo)²⁴⁾.

1) ARMADA study: An MTX add-on trial

Two hundred seventy-one patients with active RA were randomly assigned to four treatment arms: placebo + MTX or doses of 20 mg, 40 mg, or 80 mg of adalimumab every other week over the course of 24 weeks⁴⁰⁾. The patients had active RA even though a sufficient dose of MTX (12.5-25 mg/week or 10 mg if intolerant to higher doses) had been administered for at least 6 months. The adalimumab-treated groups exhibited significantly higher ACR20% and ACR50% response rates than the placebo group⁴⁰⁾. Furthermore, the ACR70% rate in the 40-mg and 80-mg groups was significantly higher than in the placebo group. These results indicate a rapid and sustained improvement over 24 weeks in the adalimumab groups, compared with placebo + MTX group; however, the effect on radiographic progression was not documented in this trial.

2) PREMIER study: Comparison of MTX, adalimumab, and MTX + adalimumab in patients with early RA

Among 799 patients with early active RA (less than 3 years), patients treated with MTX + adalimumab (40 mg every other week) exhibited a significantly higher ACR50% response than those treated with MTX or adalimumab alone (62% vs. 46% or 41%, respectively) after 1 and 2 years; the ACR20%, ACR70%, and ACR90% responses were also superior after 1 and 2 years. Significantly less radiographic progression was observed in the combined treatment group (1.3 and 1.9 sharp units at 1 and 2 years, respectively), compared with the MTX-alone group (5.7 and 10.4 sharp units at 1 and 2 years, respectively) or the adalimumab-alone group (3.0 and 5.5 sharp units at 1 and 2 years, respectively). These results indicate that the combination of TNF inhibitors and MTX appears to be a powerful tool for retarding joint destruction⁴¹⁾.

Safety

Generally, biological agents are well tolerated and safe for most patients. Frequent but minor adverse effects that can be alleviated with appropriate management have been reported. While infliximab has been used in over 500,000 patients and etanercept has been used in 200,000 patients worldwide, few but severe adverse effects have been reported. In addition, post-marketing surveys have been conducted for all patients with RA who were treated with infliximab or etanercept in Japan. The survey

on infliximab use included 5,000 cases over a 6-month period and concluded that infliximab was safe for use in Japanese patients with RA. The survey on etanercept use in Japan is ongoing. To promote the knowledge of Japanese physicians and rheumatologists regarding the indications and contra-indications for infliximab and etanercept use, guidelines for the use of anti-TNF biologics have been released and were utilized in a post-marketing survey conducted in Japan (Table 2 and 3)^{42,43)}. Individual adverse effects that have been reported worldwide, but particularly in Japan, as part of post-marketing surveys are listed below.

1) Infusion or injection reactions

Infliximab has been associated with infusion reactions, which rarely include severe events such as life-threatening anaphylaxis reactions. The lower the dose of MTX or infliximab, the higher the rate of HACA production⁴⁴⁾. In previous studies, the minimum dose of MTX was 7.5 mg/week and that of infliximab was 3mg/kg in Western countries, enabling sufficient prophylaxis against HACA production. In clinical trials in Western countries, the frequency of infusion reactions has been reported to be around 5%⁴⁵⁾, while that of a placebo infusion was 2.0%. Patients with infusion reactions leading to the withdrawal of treatment accounted for 2.5% of all patients, and those with serious infusion reactions accounted for 0.8-3%. HACA can be detected in up to 10% of all patients, even with the concomitant administration of MTX, at doses of more than 7.5 mg/week, which are sometimes used in Western countries. There was some concern that the frequency of infusion reactions and HACA production might be higher in Japan, since the average dose of MTX used in Japan is much lower than that used in Western countries. However, the frequency of infusion reactions in Japanese PMS study was 9.7%, which is comparable to those reported in Western countries. These results suggest that the minimum weekly MTX dose of 6 mg used in the Japanese clinical trial may have had an immunosuppressant effect similar to those of doses used in Western countries.

On the other hand, injections of etanercept may lead to local reactions in 40-45% of patients⁴⁶⁾. These reactions are usually mild, and most of them appear to resolve spontaneously or with appropriate management, such as varying the injection site or administering anti-histamines. Anti-neutralizing antibodies have never been observed, although anti-etanercept antibodies have been detected to some extent.

Interestingly, HAMA or anti-idiotypic antibodies have been detected in 1-12% of patients treated with adalimumab. Furthermore, anti-neutralizing antibodies have been observed with a frequency of around 5%, in spite of the fact that adalimumab is a fully human genetically engineered product. Concomitant use

Table 2 Guidelines for the use of infliximab for RA therapy in Japan

Indications:

Active RA despite previous treatment with MTX (Rheumatrex) (= 3 months with MTX = 6 mg/w). The following 3 items should be satisfied:

1. = 6 painful joints
2. = 6 swollen joints
3. CRP = 20 mg/L or ESR = 28 mm/hr

For patients with a low risk of opportunistic infections, the following 3 items should be satisfied:

1. WBC = 4000/mm³
2. Total lymphocyte count = 1000/mm³
3. β -D-glucan negative

Contraindications:

1. Active infectious disease
2. History of a serious infection during the past 6 months
3. Chest X-rays showing old TB (e.g. Calcification shadow = 5 mm
4. Latent TB. For cases where the benefit outweighs the risks, Remicade may be considered after treatment for TB.
5. History of ex-pulmonary TB, Carinii pneumonia
6. Congestive heart failure
7. Malignant tumor, demyelinating disease

Cautions:

A. The following items are important because of infection screening (TB, opportunistic infections) and adverse effects:

1. Chest X-rays should be taken on the same day and should be read by a pulmonologist or radiologist.
2. Opportunistic infections can be treated. Evaluate risk through an interview, tuberculin test, chest X-ray, and chest CT scan, etc.
- B. Precautions against infusion reactions (including anaphylactic reactions) are required.

The institution should be able to carry out emergency treatments: maintain an airway, administer oxygen, epinephrine, corticosteroids, etc.

*: refer to the next page for the treatment of infusion reactions.

Table 3 Treatment guidelines for the use of etanercept in Japan

A. Inclusion criteria

Patients with active rheumatoid arthritis and presenting the following criteria despite the use of one or more MHLW-recommended ADMARDs (methotrexate, bucillamine, or sulfasalazine) at a normal dose for more than 3 months:

- (1) Tender joints ≥ 6
- (2) Swollen joints ≥ 6
- (3) ESR ≥ 28 mm/h or CRP ≥ 2.0 mg/dl

Also, the patients must meet the following criteria to insure a low risk of opportunistic infections:

- (1) WBC $\geq 4000/\text{mm}^3$
- (2) Peripheral blood lymphocytes $\geq 1000/\text{mm}^3$
- (3) Serum β -d-glucan: negative

B. Usage

The dose of etanercept is 10 – 25 mg administered twice weekly as a subcutaneous injection. A patient can self-inject etanercept only after the ability to self-inject is carefully assessed and appropriate training is provided by a health professional.

(Additional Information)

While etanercept monotherapy has proven effective in overseas/domestic clinical trials, Western data indicates that the combination of etanercept and methotrexate has a greater efficacy than etanercept alone. Therefore, the concomitant use of methotrexate should be considered according to the course of the disease in the patient. The combination of etanercept plus methotrexate is especially recommended when the normal dose of methotrexate failed.

C. Contraindications

1. Ongoing infection
2. Past history of serious infections in the last 6 months
3. Abnormal shadows on chest radiographs suggestive of old pulmonary tuberculosis (TB) or tuberculosis pleuritis
4. History of extra pulmonary TB or *Pneumocystis carinii* pneumonia
5. Congestive heart failure
6. Malignancy or demyelinating disease

D. Cautions

1. From the point of view of screening for infection (especially TB and opportunistic infections) as well as the prevention of adverse effects, etanercept is recommended for clinical use at medical institutes where:

- (1) Chest X-rays can be obtained on the same day and the X-ray can be interpreted by a pulmonologist, TB specialist, or radiologist
- (2) Opportunistic infections can be treated
2. Comprehensive TB screening should be conducted, including an in-depth patient history, chest radiographs (chest CT whenever possible) and a PPD skin test. In patients with suspected TB, based on medical history, abnormal shadows on chest radiographs suggestive of old pulmonary TB or with a PPD skin test positive (as evidenced by redness of at least 20 mm in diameter or the presence of induration), treatment with etanercept may be considered in addition to anti-tuberculosis drugs only if the potential benefits outweigh the potential risks.

of MTX reduces the frequency, as in the case of infliximab.

2) Infections

A series of basic experiments have indicated that the blockade of TNF- can result in serious infectious diseases caused by intra-cellular pathogens like mycobacterium^{47,48)}, *Pneumocystis*

*carinii*⁴⁹⁾, other fungi⁵⁰⁾, legionellae⁵¹⁾, and *Listeria monocytogenesis*⁵²⁾.

In the ATTRACT study, the frequency of infection was 40% in the MTX-alone group and 50% in the MTX + infliximab group, although this difference was not statistically significant⁵³⁾.

The frequencies of serious infections among patients treated with MTX alone and those treated with MTX + infliximab were almost the same. Similarly, the rate of infection in etanercept-treated patients was 50-60%, which was similar to the rate in the control population⁵³. Again, the risk of serious infection was comparable between the general population and the etanercept-treated patients. However, the incidence of severe infection in patients treated with anti-TNF biologicals in clinical settings, though not in clinical trials, has been reported to be higher in patients with fewer signs and symptoms^{54,55}.

However, a post-marketing survey revealed that the incidence of tuberculosis was relatively high in RA patients treated with MTX + infliximab, particularly in Europe^{56,57}. Although the incidence of tuberculosis in RA patients treated with etanercept appears to be at the upper limit of the normal controls⁵⁸, a risk of tuberculosis has been reported in patients treated with adalimumab. Since infliximab and adalimumab are believed to lyse TNF-expressing macrophages (Fig.4), which play an indispensable role in host defense against mycobacteria infections, the incidence may be higher in patients treated with infliximab and adalimumab. Considering the relatively high risk of tuberculosis, a Tuberculin skin test has been recommended to rule out latent tuberculosis infection⁵⁸. Following the implementation of this screening procedure, the incidence of tuberculosis in patients being treated with anti-TNF biologicals reportedly decreased⁵⁷. Given the high incidence of tuberculosis in Japan, with 30 out of 100,000 subjects contracting the disease in 2001 (one of the highest rates among industrialized nations, where the rate is typically less than 10 in 100,000), the increased risk of tuberculosis in this patient population (up to 7×30 in 100,000) is an important concern. To this end, guidelines for the prescription of infliximab in Japan have been proposed, and these guidelines were revised when etanercept was approved for the treatment of RA in March 2005 (Table 2). In spite of precautions regarding the increased risk of tuberculosis among RA patients treated with TNF inhibitors, 11 patients out of 2000 initial cases treated with infliximab developed tuberculosis; all 11 of these cases had not received INH (Isoniazid) prophylaxis. After an intense effort to screen patients and provide INH prophylaxis in high-risk cases, the incidence of tuberculosis has decreased. In contrast, 22 cases of *Pneumocystis jirovecii* (formerly *carinii*) pneumonia were reported among 5000 cases treated with infliximab. Although the condition of these patients was resolved after appropriate treatment, the possibility of other opportunistic infections, such as those caused by fungi⁵⁹, in addition to mycobacteria infections must be carefully considered, and similarities and dissimilarities in the incidences of opportunistic infections should be compared between Western countries and Japan.

3) Autoimmunity

Infliximab and etanercept can reportedly induce positivity for anti-nuclear antibodies and anti-DNA antibodies, although the titers of the antibodies remain low. In earlier studies, an increased risk of developing SLE (systemic lupus erythematosus) had been seriously considered. However, the incidence of this disease has not been significantly high in the treatment group, and SLE is not a severe life-threatening disease, even if it does develop²². On the other hand, the risk of demyelinating diseases such as multiple sclerosis or optic neuritis appears to be high, and TNF blockade therapy is contra-indicated in patients with these diseases. Curiously, a recent clinical trial for chronic inflammatory demyelinating diseases suggested a significant clinical response⁶⁰. Changes in the cytokine environment, which is largely skewed by anti-TNF biologicals, is believed to account for the change in the profile of autoantibody production and the phenotypes of autoimmune diseases.

4) Malignancies

As mentioned above, TNF- α induces tumor cell lysis *in vitro*. Thus, the possibility that a blockade of TNF- α may result in an increased incidence of malignancy must be considered. So far, the incidence of non-hematological malignancies appears to be comparable with that of the general population, while that of malignant lymphoma may be higher in patients treated with anti-TNF biologicals⁶¹. Since the incidence is clearly higher in patients with more active RA and in MTX users, the risk may not be simply attributed to the use of biologicals alone. The incidence of malignancies must be carefully followed over an extended period.

5) Congestive heart diseases

Since TNF- α is thought to have a role in the pathogenesis of heart failure, clinical trials for infliximab and etanercept have been conducted in patients with congestive heart failure. In contrast to earlier expectations, the mortality rate of patients treated with higher amounts of infliximab was higher than that of the placebo group, and no significant benefit was reported in the etanercept-treatment group. These results indicate that anti-TNF biologicals are not appropriate for patients with congestive heart failure²².

Future perspectives

The usefulness of anti-TNF biologicals in combination with MTX or other biologicals clearly lies not only in the induction of clinical remission, but also in the arrest of structural damage in early RA³⁹. These results have led to the introduction of a new strategy whereby powerful combination therapy is applied to early RA as an induction regimen. To take advantage of this strategy, we need to be able to identify patients who have a poor

prognosis and to perform appropriately powerful treatments.

Given that biologicals targeting TNF, IL-1 and IL-6 as cytokines and CD20 and CTLA-4 as cell surface structures are now available, we now must be able to predict responders to each biological before treatment or early after the start of treatment to avoid toxicity in non-responders and to provide resources efficiently to patients who will benefit from the treatments. If predictive markers can be identified, tailor-made therapies using several biologics may soon become a clinical reality.

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