

## **TNF and TNF $\alpha$ Inhibitors: Mechanisms of action**

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## **Abstract**

Inhibition of TNF $\alpha$  has become almost commonplace within the past few years. In spite of the popularity of TNF $\alpha$  inhibitors, we have an incomplete understanding of their modes of action. This review will discuss the well-known effects of TNF $\alpha$  and recent hypotheses regarding its actions. The best known effects of TNF $\alpha$  relate to its proinflammatory effects such as induction of adhesion molecules and stimulation of cytokine and chemokine production. Less well known is the fact that TNF $\alpha$  can also have immunosuppressive effects such as inhibition of T cell receptor signaling and the induction of immunosuppressive cytokine expression. Just as TNF $\alpha$  has a broad range of effects, inhibition of TNF $\alpha$  leads to numerous changes in immunologic function.

## Clinical examples of TNF $\alpha$ over-production

TNF $\alpha$  over-expression has been documented in a number of inflammatory processes which led to the first successful attempts to block a cytokine therapeutically. A monoclonal antibody to TNF $\alpha$  or infliximab, was originally piloted in rheumatoid arthritis and Crohn's disease. In both cases the therapeutic effect was dramatic with dose-dependent clinical and laboratory responses. The original rationale for the use of antibodies to TNF $\alpha$  in rheumatoid arthritis was the finding that the two predominant cytokines in synovial fluid are IL-1 and TNF $\alpha$  [1]. These two cytokines act both directly and indirectly. For example, IL-1 and chemokine production are driven largely by the TNF $\alpha$ . TNF $\alpha$  acts directly by promoting the release of metalloproteinases and leukotrienes which are responsible for tissue damage. In Crohn's disease, TNF $\alpha$  is also elevated, with lamina propria T cells appearing to be largely responsible [2, 3]. This is supported by murine studies of TNF $\alpha$  over-expression. When over-expression is limited to monocytes and macrophages, the phenotype is limited to arthritis and dermatitis. The inflammatory bowel disease phenotype occurs with T cell production of TNF $\alpha$  {Kontoyiannis, 1999 #3479}{Kontoyiannis, 1999 #3479} [4].

While Crohn's disease and rheumatoid arthritis were the first two disorders in which TNF $\alpha$  inhibition was tested therapeutically, there are now many other disorders in which TNF $\alpha$  inhibition has been tried and found effective (Table 1). These disorders are surprisingly diverse and the exact mechanism by which the TNF $\alpha$  inhibitor is acting is not always known.

While it would be tempting to administer TNF $\alpha$  inhibitors in any setting where TNF $\alpha$  over-expression is documented, caution is warranted as TNF $\alpha$  inhibition has been shown to worsen multiple sclerosis and can induce anti-dsDNA antibodies [5, 6]. In addition, the known risks of infection with intracellular organisms such as *Salmonella*, *Listeria*, *Histoplasma*, *Mycobacteria*, and *Toxoplasma* are becoming increasingly recognized. Individual reports of patients with viral infections, sepsis, thrombosis, heart failure, liver failure, and lymphoma also suggest that significant caution is warranted when using TNF $\alpha$  inhibitors. Understanding the mechanism of action of TNF $\alpha$  and the effect of inhibition can clarify some of the clinical effects that have been seen.

## Mechanisms of action

### Rheumatoid arthritis.

Inflammation is often thought of as an aberrant over-response of the innate immune

system. Studies of murine models and *in vitro* organ culture reveal that inflammation is a complex process which may differ in different organs. An elaborate interaction between the innate and adaptive immune responses is required to sustain an inflammatory response. The best characterized example is the rheumatoid synovium. When actively inflamed, activation of T cells, B cells, macrophages, fibroblasts, endothelial cells and plasma cells can be identified. Pro-inflammatory cytokines are spontaneously produced by explant cultures [1]. Specifically, IL-1, TNF $\alpha$ , lymphotoxin, IL-6, GM-CSF, LIF, IL-12, IL-15, IL-18, and a range of chemokines have been identified. To counter this enormous load of pro-inflammatory cytokines, a number of anti-inflammatory mediators are up-regulated such as IL-10, IL-11, IL-1RA, and other soluble receptor antagonists [7]. The anti-inflammatory cytokines and receptors are insufficient to block the action of the pro-inflammatory cytokines and this leads to fatigue, fever, elevation of acute phase proteins, angiogenesis, bone marrow suppression, increase in adhesion molecules on endothelium, activation of macrophages, and induction of metalloproteinases and leukotrienes (Figure 1). All of these effects contribute to active rheumatoid arthritis. TNF $\alpha$  is a major mediator of both primary and secondary cytokine and chemokine effects. Understanding that TNF $\alpha$  is responsible for so many of the inflammatory pathways activated in rheumatoid arthritis was critical to the development of this important therapeutic strategy (Figure 2). Neutralization of TNF $\alpha$  in synovial organ cultures reduces IL-1, GM-CSF, IL-6, and IL-8 production. This effect is unidirectional because IL-1 inhibition diminishes IL-6 and IL-8 production but not TNF $\alpha$  production suggesting that TNF $\alpha$  is the predominant mediator of rheumatoid synovial inflammation [8].

### **Juvenile chronic arthritis.**

Although juvenile chronic arthritis (JCA) has many similarities with rheumatoid arthritis, the pathologic processes are distinct. TNF $\alpha$  over-expression is implicated specifically in all forms of JCA although each type of arthritis has different cytokine profiles, different pathology, and different natural history.

Systemic JCA has been extensively evaluated for cytokine abnormalities. IL-6, IL-18, IL-8, monocyte chemoattractant protein-1, and migration inhibitory factor have all been found to be markedly elevated in the serum of patients [9-12]. This elevation of IL-6 may in part mediate the angiogenesis and growth failure seen in systemic JCA [13, 14]. TNF $\alpha$  is elevated in synovial fluid, and serum levels are extremely high in patients with macrophage activation syndrome. TNF $\alpha$  inhibitors have been rarely successful in patients with systemic JCA; however, the

response is often poor. There is currently a trial using an antibody to IL-6 which is showing early promise [15].

The synovium in pauciarticular and polyarticular JCA can appear quite similar histopathologically; therefore, it is not surprising that the cytokines found in the synovial fluid are similar.  $\text{TNF}\alpha$ , IL-1, IL-12, monocyte chemoattractant protein-1, interferon- $\gamma$ , IL-18 and IL-15 are overproduced locally [10, 16, 17]. One significant difference between pauciarticular and polyarticular synovial cytokine expression is that pauciarticular JCA is associated with increased IL-4 expression while polyarticular synovial samples are not [18]. Thus, the secretion of  $\text{TNF}\alpha$  in the synovium is a commonality exploited therapeutically. The mechanism of action in JCA may, in fact, be quite similar to that described for rheumatoid arthritis.

### **Systemic effects.**

$\text{TNF}\alpha$  has numerous systemic effects. High levels of circulating  $\text{TNF}\alpha$  are known to impair T cell responses and treatment with  $\text{TNF}\alpha$  inhibitors has been shown to restore T cell function [19]. Aberrant cytokine production by T cells also normalizes after treatment [20]. Furthermore, a recent study in mice demonstrated that the production of the CD4/CD25 T cells in the thymus is impaired in the presence of elevated  $\text{TNF}\alpha$  and is improved by administration of anti-  $\text{TNF}\alpha$  antibodies [21]. These CD4/CD25 T cells have been shown to be critically important for the prevention of organ-specific autoimmunity in mice and appear to have a similar role in humans [22]. Other systemic effects have been noted during treatment with  $\text{TNF}\alpha$  inhibitors. Endothelium-dependent vasodilation improved in patients treated with  $\text{TNF}\alpha$  inhibitors and endothelial cell expression of adhesion molecules diminished [23]. Macrophage activation was diminished and less nitric oxide was produced after treatment [24, 25]. Bone marrow suppression was reversed and angiogenesis impaired. Thus,  $\text{TNF}\alpha$  inhibition acts not only in the joint space to suppress inflammation but globally resets the immune system in a favorable way and suppresses the systemic symptoms associated with chronic inflammation such as fatigue and fever.

### **Mechanisms of adverse events**

The features described above all contribute to therapeutic efficacy. The adverse effects associated with its use can also be understood by examining the mechanisms of  $\text{TNF}\alpha$  action. The risk of infection with intracellular organisms is clearly increased in patients treated with

TNF $\alpha$  inhibitors and this risk is derived from the role TNF $\alpha$  plays in the activation of intracellular killing in macrophages. Interferon-g, TNF $\alpha$  and various chemokines participate in granuloma formation which serves to contain and suppress intracellular organism growth. TNF $\alpha$  is also required for intracellular killing of pathogens. Figure 3 demonstrates the cytokine network that governs intracellular killing. It may be seen that TNF $\alpha$  is essential to the process. Thus, it is no surprise that clinical inhibition of TNF $\alpha$  promotes susceptibility to intracellular pathogens. It is less clear whether inhibition of TNF $\alpha$  leads to a greater susceptibility to infection in general. Patients who are unable to respond to interferon-g due to an inherited mutation, have a slightly increased risk of infection with Herpes viruses which may be due to the effect of interferon-g on Th1 cell maturation [26]. Relatively few severe viral infections have been reported in patients receiving TNF $\alpha$  inhibitors and the expectation would be that any increase in susceptibility to viral infections would be modest and more likely to be seen in children when the first exposures to Herpes family viruses occur.

The risk of bacterial infections is almost certainly increased in patients receiving TNF $\alpha$  inhibitors, although clinical data has been difficult to collect. TNF $\alpha$  is released from neutrophils and macrophages upon first encounter with bacterial pathogens. Recognition of pathogens via toll-like receptors leads to TNF $\alpha$  expression along with other proinflammatory cytokines [27]. Early expression of cytokines and chemokines is important to upregulate adhesion molecules to bring other responding cells to the site [28]. TNF $\alpha$  expression also leads to activation of macrophages which improves phagocytosis and antigen presentation. Thus, early TNF $\alpha$  expression is important for innate responses to infection and to improve interactions between macrophages and T cells.

There have been 170 cases of lymphoma occurring in patients receiving TNF $\alpha$  inhibitors as of March 2003 (<http://www.fda.gov/ohrms/dockets/ac/cder03.html#Arthritis>). Most cases were non-Hodgkins lymphoma. Several of these cases regressed when the patients were taken off their TNF $\alpha$  inhibitor. An interesting feature is that most lymphomas developed very soon after initiation of therapy [29]. At this point, causality has not been demonstrated because patients with autoimmune diseases have an increased risk of malignancy over the general population. Improved epidemiologic analysis should advance our understanding of the relationship of TNF $\alpha$  inhibition and lymphoma development. Nevertheless, there are theoretical reasons to believe the relationship may be real. Both T cells and natural killer cells are important in the surveillance for malignancies [30]. Figure 3 demonstrates the role of TNF $\alpha$  in

natural killer cell activation. It is possible that TNF $\alpha$  inhibition leads to impaired natural killer cell function which in turn, allows cells already undergoing malignant transformation to escape detection and proliferate. This is a topic of great importance because patients are being maintained for longer and longer periods on TNF $\alpha$  inhibitors.

## Summary

TNF $\alpha$  is well known for its proinflammatory effects and inhibition has unquestionably been an enormously beneficial strategy. Less well known are the immunosuppressive effects of TNF $\alpha$  and the deleterious consequences of TNF $\alpha$  inhibition.

## Figure Legends

Figure 1. The roles of TNF $\alpha$  in inflammation and immunosuppression. TNF $\alpha$ , like most members of the TNF family of proteins, has seemingly contradictory roles. It plays important roles in driving inflammation but also has immunosuppressive effects such as bone marrow suppression, induction of apoptosis and inhibition of dendritic cell function.

Figure 2. TNF $\alpha$  stimulates the production of many cytokines. Many of the inflammatory effects of TNF $\alpha$  can be understood by examining the cytokines and chemokines induced in the presence of TNF $\alpha$ .

Figure 3. The role of TNF $\alpha$  in the defense against intracellular organisms and malignancy. TNF $\alpha$  is part of a circuit of cytokines that form the basis for innate responses to infection. Through increasing uptake and killing of microbes, TNF $\alpha$  augments innate defenses. Through increasing co-stimulatory molecule expression and expression of major histocompatibility proteins, TNF $\alpha$  also stimulates the adaptive immune response. Its actions on natural killer cells may augment killing of transformed target cells and the defense against viral infections.

**Table 1****Autoimmune disorders treated with TNF $\alpha$  inhibitors**

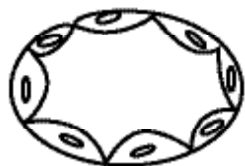
| <b>Disorder</b>                                   | <b>Type of study*</b> | <b>References</b> |
|---|-----------------------|-------------------|
| Rheumatoid arthritis                              | PC                    | [31-33]           |
| Crohn's disease                                   | PC                    | [34, 35]          |
| Spondyloarthropathy                               | PC, OL                | [36, 37]          |
| Juvenile rheumatoid arthritis                     | PC                    | [38]              |
| Psoriasis (skin and arthritis)                    | PC                    | [39, 40]          |
| Wegener's granulomatosis                          | OL                    | [41]              |
| Adult onset Still's disease                       | OL                    | [42-44]           |
| Behcet's  | OL                    | [45]              |
| AA amyloidosis                                    | OL                    | [46]              |
| Pyoderma gangrenosum                              | OL                    | [47, 48]          |
| Chronic inflammatory demyelinating polyneuropathy | OL                    | [49]              |
| Polymyalgia rheumatica                            | OL                    | [50]              |
| Uveitis   | OL                    | [51]              |
| Chronic ITP                                       | OL                    | [52]              |
| Reactive arthritis                                | OL                    | [53]              |
| TNF receptor associated periodic syndrome         | OL                    | [54]              |
| Ulcerative colitis                                | OL                    | [55, 56]          |
| Sjogren's syndrome                                | OL                    | [57]              |
| SAPHO syndrome                                    | OL                    | [58]              |
| Sarcoidosis                                       | OL                    | [59]              |

\*OL=Open label, PC= placebo controlled

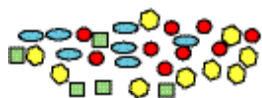
**Proinflammatory effects**



**Activation of macrophages**  
 Increased antigen presentation  
 Increased adhesion molecules  
 Increased phagocytosis/killing



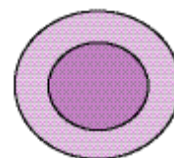
**Activation of endothelium**  
 Increased adhesion molecules  
 Aberrant vasodilation



**Production of inflammatory mediators**  
 Chemokines  
 Leukotrienes  
 Reactive oxygen species  
 IL-6, IL-1, IL-12



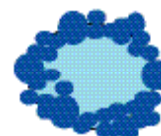
**Immunosuppressive effects**



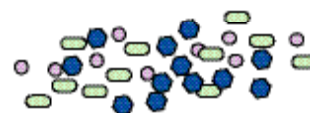
**Inhibition of T cell function**



**Inhibition of dendritic cell function**

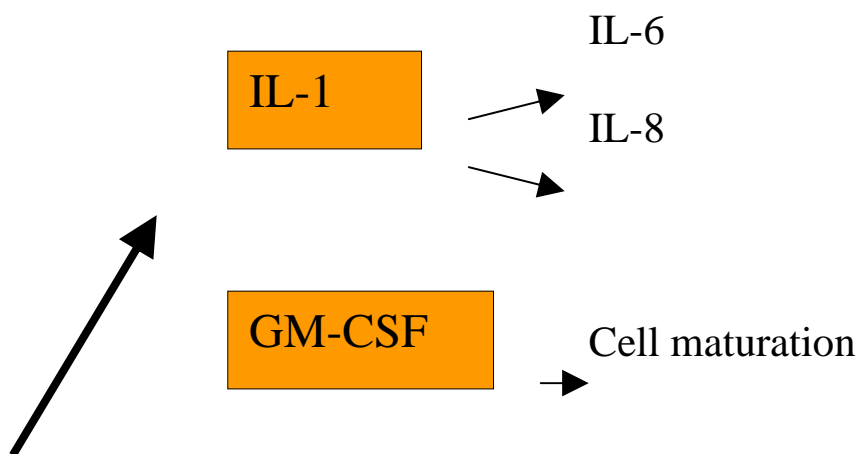


**Apoptosis and suppression of growth**



**Anti-inflammatory cytokines**  
 TGF-β, IL-10

Figure 1



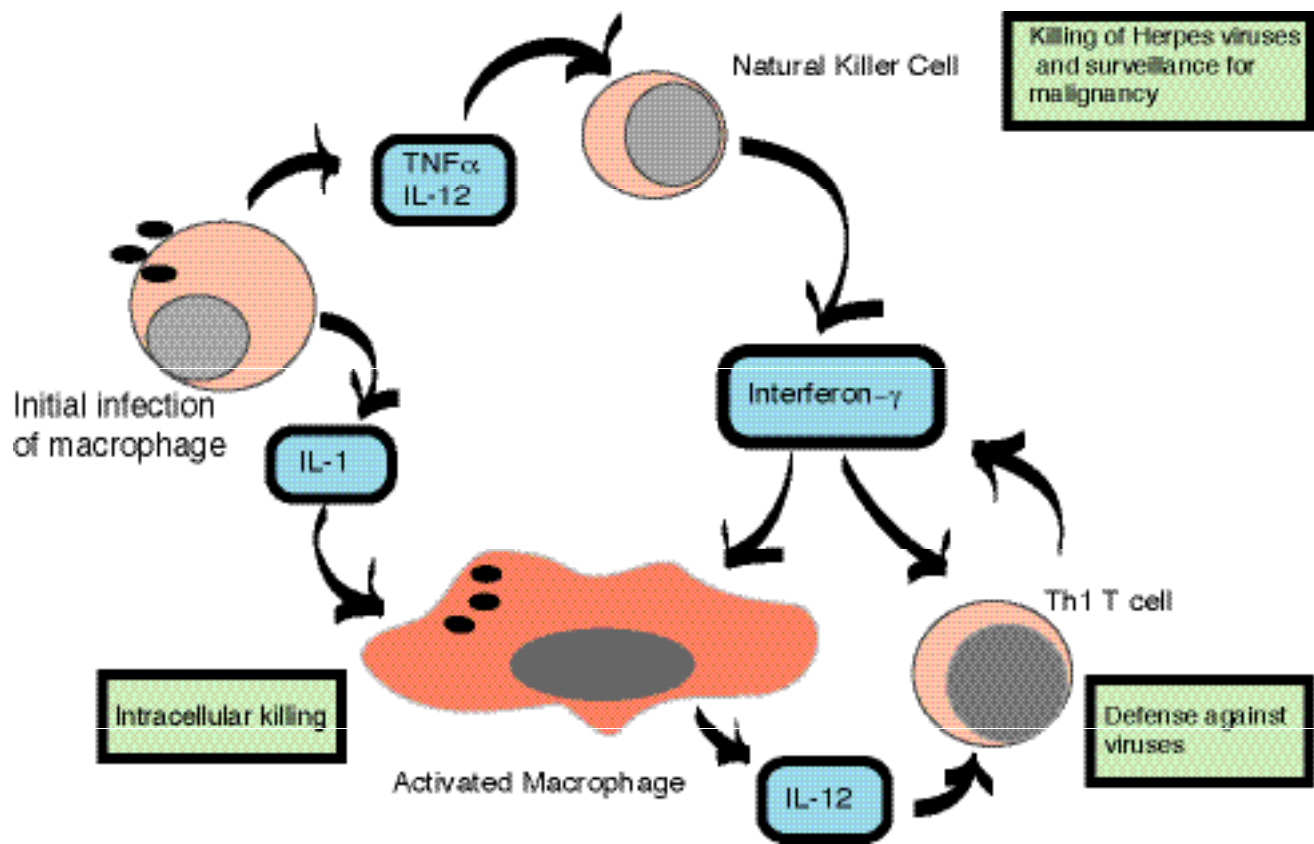


Figure 3

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