Differences Between Anti-Tumor Necrosis Factor-α Monoclonal Antibodies and Soluble TNF Receptors in Host Defense Impairment

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ABSTRACT. This review examines the differences in the incidence, spectrum, and mechanisms of activation of opportunistic infections, such as *Mycobacterium tuberculosis*, in patients with rheumatic diseases treated with the soluble TNF p75 receptor etanercept compared to the 2 anti-TNF-α monoclonal antibodies, infliximab and adalimumab. (J Rheumatol 2005;32 Suppl 74:40-47)

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INTRODUCTION

Impairment in host defense against infection and cancer is a broad topic that includes various clinical and biological conditions that are either innate or acquired. Both types of impairment, however, can result in similar clinical outcomes such as infection due to intracellular microorganisms and increased lymphoma, particularly Epstein-Barr virus.

Similarities in the spectrum of infectious diseases in persons born with defects in CD4+ T cell functions and those who lose CD4+ T cell numbers associated with infection with HIV-1 are well documented. Although longterm immunosuppressive drug therapy in patients with organ transplants is a source of epidemiologic data, in this review, there will be no discussion of impaired host defense associated with immunosuppressive agents. The agents used to suppress the immune response in these patients are nonspecific, in that more than a single cytokine is affected. In patients receiving tumor necrosis factor (TNF) blocking agents for chronic diseases such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), psoriatic arthritis, or psoriasis, the specificity of anti-TNF-α monoclonal antibody has important implications because only TNF-α or cytokines related to TNF-α activity are affected.

Although the soluble TNF receptors (etanercept, onercept, pegsunercept, lenercept) share with anti-TNF-α monoclonal antibodies the ability to neutralize both extracellular as well as membrane forms of TNF-α, these soluble receptors also can bind and neutralize TNF-β (lymphotoxin), which is produced by macrophages as well as T cells. Given the number of cytokines that are implicated in host defense functions, the remarkable lesson from the use of TNF blockers in hundreds of thousands of patients reinforces the importance of TNF in host defense. However, of equal importance is the clinical finding that differences exist in the incidence and spectrum of infections in patients treated with the soluble TNF p75 receptor etanercept compared to either of the 2 anti-TNF-α monoclonal antibodies, infliximab or adalimumab.

In a recent review of the incidence of granulomatous infections reported to the US Food and Drug Administration from 1998 to September, 2002, there were 239 per 100,000 infliximab-treated patients compared to 74 per 100,000 for etanercept-treated patients[1]. *Mycobacterium tuberculosis* infections occurred at rates 5 times greater for infliximab than for etanercept[1]. Of these, the onset of *M. tuberculosis* reactivation occurred in 72% of patients within 90 days of initiating infliximab compared to 28% for etanercept[1]. In a correction to the published analysis[2], the number of patients in the USA treated with infliximab was reduced from 233,000 to 197,000, and the rate of *M. tuberculosis* infection associated with infliximab was 54 per 100,000 patients compared to 28 per 100,000 for etanercept. However, upon reanalysis of the reported cases, the rate of reactivation of *M. tuberculosis* infections within the first 90 days of treatment was 95 per 100,000 patient-years for infliximab compared to 11 per 100,000 patient-years for etanercept[2]. Therefore, these data continue to reveal a difference between *M. tuberculosis* infections associated with anti-TNF-α monoclonal antibody and those associated with soluble TNF receptors.

Although there are no comparable studies of reactivation of *M. tuberculosis* infections in patients receiving adalimumab using comparable numbers of patient-years of treatment, there were 13 cases of tuberculosis in 2400 treated patients in controlled studies of this antibody,

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which resulted in a boxed warning added to the adalimumab label[3].

Opportunistic infections associated with TNF blockers, particularly with anti-TNF-α monoclonal antibodies, are often similar to infections observed in immunosuppressed patients, particularly in HIV-1-infected patients. For example, about 50% of *M. tuberculosis* infections are extrapulmonary in HIV-1 patients, and similar percentages have been reported for patients treated with TNF blockers[3]. However, in the case of etanercept, the safety of this TNF blocker was evaluated in HIV-1 patients with known *M. tuberculosis* infection being treated with anti-tuberculous therapy. Eight doses of etanercept were administered during initiation of anti-tuberculous therapy, and improved clinical responses were reported for patients receiving etanercept compared to those without etanercept[4]. Indeed, CD4+ T cell counts increased associated with etanercept therapy. Other studies in patients with AS being treated with infliximab or etanercept[5,6] support the concept that the mechanism of action of the monoclonal antibody and the soluble TNF receptor are different and account for the differences in the clinical spectrum of diseases associated with impaired host defense.

CYTOKINES DID NOT EVOLVE AS MEDIATORS OF DISEASE

Prototypes of cytokines such as interleukin 1 (IL-1) or TNF-α and their respective signaling mechanisms can be found in insects; indeed, nuclear factor-κB and the signal-transducing domains of the mammalian IL-1 receptor are found in Drosophila[7]. With the use of anticytokine based therapies, there is little question that these and other cytokines play an important role in disease. However, proinflammatory cytokines did not evolve to harm, but rather to benefit the host. Other cytokines such as IL-18 and IL-15, which also play a role in autoimmune diseases, similarly evolved as beneficial to the host, particularly in terms of host defense to infection. Indeed, the use of TNF-blocking therapies has revealed the importance of this cytokine for host defense in humans.

The level of risk to the host, however, remains controversial because of absence of controlled data to determine: (1) the incidence of infections in patients treated with immunosuppressive agents, such as low dose corticosteroids and/or methotrexate, without TNF-blocking therapies; (2) the number of patient-years of therapy used to calculate the denominator; and (3) the true number of the numerator when reporting of infection is voluntary. In the case of infliximab, a single injection of the antibody is carried forward and added to the database for number of patient-years of treatment, whereas this is not the method used to calculate the database for anakinra or etanercept.

In general, a host challenged with infection musters the assistance of these cytokines to stop the microbe from spreading and to eliminate infection by microbicidal mechanisms. For non-intracellular bacteria, the effector cell is the phagocyte, most notably the neutrophil. These cells have the machinery to engulf the organism and kill it in the phagolysosome. Gram-positive bacteria such as streptococci and staphylococci are examples of neutrophil- and cell adhesion molecule-dependent host defense. In the case of macrophage intracellular microorganisms, the killing mechanisms of the infected macrophage involves interferon-γ (IFN-γ) and nitric oxide production. However, obligate intracellular organisms such as *M. tuberculosis*, Listeria, Histoplasma, and Salmonella can survive and replicate inside the macrophage by usurping killing. Most notably, cytokines such as TNF-α and IFN-γ increase the killing mechanisms, and a balance is achieved whereby a microorganism such as *M. tuberculosis* can survive inside a macrophage. Further spread and overt disease is kept to a minimum by a competent immune system and the formation of granulomata. Reducing TNF-α activity impairs the host's mechanisms of killing intracellular organisms by reducing the synergism of TNF-α plus IFN-γ, which regulate nitric oxide production. In animal models of mycobacterial infections, anti-TNF-α prevents formation of granulomata.

LIMITED ACTION OF CYTOKINES DURING INFECTION

Cytokines act at several levels in combating infection. First, cytokines such as TNF-α induce expression of endothelial and cellular adhesion molecules to facilitate adherence of phagocytes to cell surfaces. This is an important first step for emigration of phagocytes from the circulation into the site of infection. Next, increasing the production of local chemokines results in the movement of the phagocyte into infected tissue. By stimulating nitric oxide production by the phagocyte, cytokines provide the chemical agents for killing the microbe. Cytokines such as IL-1 and TNF-α are expressed during this time of burgeoning infection; however, production of cytokines is tightly regulated such that when the infection is cleared, production of these proinflammatory cytokines stops. Thus, during infection there is a rapid "on" signal for cytokine production, but after the infection, a rapid "off" mechanism limits the production of cytokines, thereby reducing any negative effect of cytokine-mediated inflammation.

In the case of a chronic disease, the activator signal of the cytokine cascade stays in the "on" mode. As a result, there are increasingly greater amounts of cytokines produced day after day and year after year. It appears that in many autoimmune diseases, the production of these cytokines is dysregulated by the disease processes. The most likely regulator of the disease process is an ongoing autoimmune process. Proinflammatory cytokines such as TNF-α function as a mediator of the disease, not the cause of the disease, since upon cessation of TNF- blocking therapy, the disease returns.

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For the patient, the aim of therapy is to block the inflammatory effects of these cytokines. For a disease such as RA, the therapeutic effect is a reduction in cellular migration into the joint space. Although decreased cell adhesion, cellular infiltration, and a reduction in the inflammatory mediators such as nitric oxide reduce host defense, the net effect of anticytokine therapy appears to benefit the host. This may be because simple pharmacokinetics limits the duration of depressed host function and allows the host's mechanisms for fighting infection to be transiently suppressed, with somewhat the same result accomplished when corticosteroids are administered once a day or every other day. One benefit of shorter-acting agents such as soluble TNF receptors is that host defense functions related to TNF return to the normal range more frequently compared to long-acting antibodies to TNF-α.

INFECTION IN PATIENTS TREATED WITH TNF BLOCKERS

Since the introduction of therapies using TNF blockers, increased infections were not unexpected. Of these, risk of serious infections with Gram-positive organisms is increased compared to Gram-negative infections. Voluntary reports of bacterial infections, however, do not reflect the true incidence because diagnosis and treatment are fairly routine. In contrast, because of their uncommon occurrence, infections with intracellular organisms are increasingly reported and analyzed. Host defenses against intracellular organisms such as Listeria[8], Histoplasma[9], Pneumocystis, or Salmonella[10] are reported with the use of infliximab. Other complications such as development of lupus-like disease, demyelinating disease, and worsening of heart failure were unexpected and, indeed, contrary to the concept of reducing TNF activity[11].

The incidence of infection in RA is increased compared to the population without the disease and is thought to be due, in the absence of treatment with agents such as prednisone, methotrexate, or cyclosporine, to the immunosuppressive component of the disease. In patients with RA treated with these agents, infections are increased further. In the case of *M. tuberculosis* in RA, more than 50% of the reported cases are of extrapulmonary and disseminated forms of infection. Most are reactivation disease and in the case of the fully human anti-TNF-α adalimumab, the effect appears to be dose-dependent[3,12]. Voluntary reports of etanercept-associated reactivation of *M. tuberculosis* are lower in number than those of the 2 monoclonal antibodies, but in the etanercept cases 50% are the disseminated disease[13]. As discussed below, treatment with etanercept is also associated with increased opportunistic fungal infections, but there are fewer voluntary reports in the postmarketing period based on numbers of patient-years of exposure.

Opportunistic infections associated with TNF blockers are similar to those that occur in HIV-1 infected persons with CD4+ T cells below 200/ml. The immunosuppression of HIV-1 infection is global but is due primarily to the loss of CD4+ T cells. It is remarkable that the specific blockade of a single cytokine (TNF-α) results in the same portfolio of infections. There are, however, some notable exceptions: in the case of TNF blocker therapies, there have been few cases of oral candidiasis, or reactivation of cytomegalovirus infection or Herpes zoster infections. These infections are common in patients with autoimmune diseases, but they have not increased to the same extent as in HIV-1 infections.

CELL DEATH BY IgG1 ANTIBODIES: IMPORTANCE OF MEMBRANE TNF-α

Fundamental to the understanding of host defense and cytokines is the concept of reversible and irreversible mechanisms of action. Soluble receptors to TNF or any monoclonal antibody to TNF-α will neutralize TNF-α activity in the extracellular space regardless of its affinity or IgG class type or whether a soluble TNF type I or soluble type II receptor. However, due to a weak signal peptide, a great deal of TNF-α is present as an intergral transmembrane protein; "membrane" TNF-α is a fully active form of the cytokine (Figure 1). Membrane IL-1α is also a fully active cytokine[14]. In the case of membrane IL-1α, the cysteine protease calpain cleaves membrane form, and mature IL-1α is released into the extracellular space. In the case of membrane TNF-α, the metalloprotease TNF-α converting enzyme (TACE) cleaves membrane TNF-α, and the mature cytokine is released into the extracellular space[15]. TACE is present in macrophages and dendritic cells and is activated in cells by various mechanisms. Activation of TACE in T cells appears to be inefficient[16]. Therefore, T cells producing TNF-α remain in the membrane form, whereas most TNF-α from macrophages is cleaved by TACE. However, as shown in Figure 1, the active TNF-α trimer can also form in the membrane as an active cytokine.

In mice with a specific deletion in the TNF-α molecule required for cleavage and secretion of TNF-α, a severe ankylosing arthritis develops by 6 weeks of age[17]. In these mice there is no TNF-α in the extracellular space and all TNF-α is present as membrane TNF-α. In addition, these mice require both the type I and type II TNF receptors for activity[18]. Therefore, "membrane" TNF-α is active by virtue of cell-cell contact. This mechanism is often called "juxtacrine." Indeed, a great deal of cytokine-mediated autoimmune and inflammatory disease is the result of cell-cell juxtacrine contact activation rather than soluble cytokines in the extracellular space. Disease in mice due to membrane TNF-α is treatable either with soluble TNF receptors or monoclonal anti-TNF-α antibodies. Indeed, in humans, a great deal of disease modification by TNF blockers is likely due to prevention of membrane TNF-α activities. In general,

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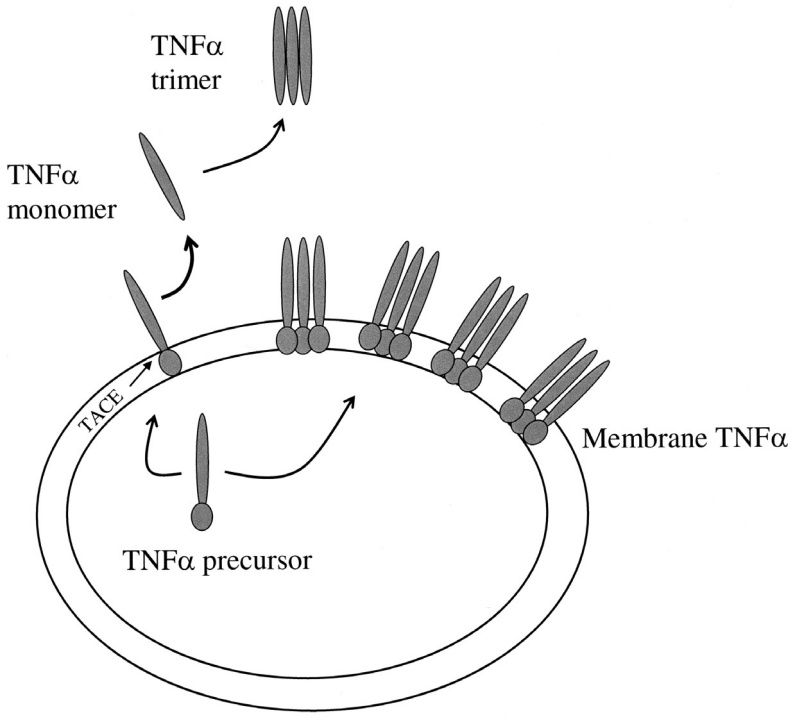

Figure 1. Extracellular and membrane TNF-α. TNF-α is initially synthesized as a precursor with a weak signal peptide for secretion. The TNF-α precursor inserts into the cell membrane and is cleaved by the TNF-α converting enzyme (TACE), a membrane associated metalloprotease[15]. The TNF-α monomer is then released from the cell and spontaneously forms a trimer (the active form of TNF-α). Alternatively, some cells without an active TACE mechanism do not cleave the membrane inserted TNF-α and there is spontaneous formation of the TNF-α trimer in the membrane[16]. This membrane TNF-α trimer is biologically active. Although all TNF-α producing cells express biologically active TNF-α trimer, macrophages have an active TACE and secrete the cytokine, whereas T cells primarily express the membrane TNF-α trimer.

CD4+ T cells as well as CD8+ T cells express primarily membrane TNF-α, whereas macrophages and dendritic cells secrete TNF-α.

TNF-α binding to the type II cell-bound receptor is rapid when compared to the binding to the type I receptor. In many ways, the same type of rapid "off" rate is observed when one considers membrane TNF-α and the binding of the type II soluble receptor. As shown in Figure 2, the soluble TNF receptor type II as the Fc bivalent construct (etanercept) binds to membrane TNF-α, but this interaction is reversible in that the inhibition of biological activity is less than that of infliximab, and the binding is of a lower affinity[19]. In contrast, infliximab forms more stable complexes with membrane TNF-α relative to the complexes formed with etanercept. Moreover, a greater number of infliximab molecules bind to membrane TNF-α with higher avidity compared to etanercept. This was supported by the observation that infliximab exhibited a greater reduction in the biological activity of membrane TNF-α–bearing cells on endothelial target cells compared to etanercept[19]. These studies are consistent with the concept that the action of etanercept on membrane as well as soluble TNF-α is "reversible," whereas the binding of monoclonal anti-TNF-α antibodies is more effective for either soluble TNF-α in the extracellular space or membrane TNF-α.

In vitro, cells expressing membrane TNF-α are vulnerable to death by either complement-mediated cell lysis or antibody-dependent cytotoxicity when exposed to anti-TNF-α (infliximab)[19,20]. The mechanism of cell death is due to cross-linking of membrane TNF-α by infliximab and activation of the so-called "membrane attack complex" by activated complement. Cell death *in vitro* is likely facilitated by the addition of exogenous complement during the assay conditions. However, clinical studies reveal a different mechanism of cell death associated with infliximab. Four hours following infusion of infliximab in patients with Crohn's disease (CD), circulating blood monocytes undergo death by caspase-dependent apoptosis[21]. In isolated lamina propria T cells from patients with CD, anti-TNF-α monoclonal antibodies of the IgG1 class result in programmed cell death (apoptosis) by a caspase-dependent mechanism[22] (Figure 3). Unlike in HIV-1 infection, these IgG1 antibodies target any cells expressing TNF-α on their membranes, including CD4+ T cells, CD8+ T cells, macrophages, and dendritic and

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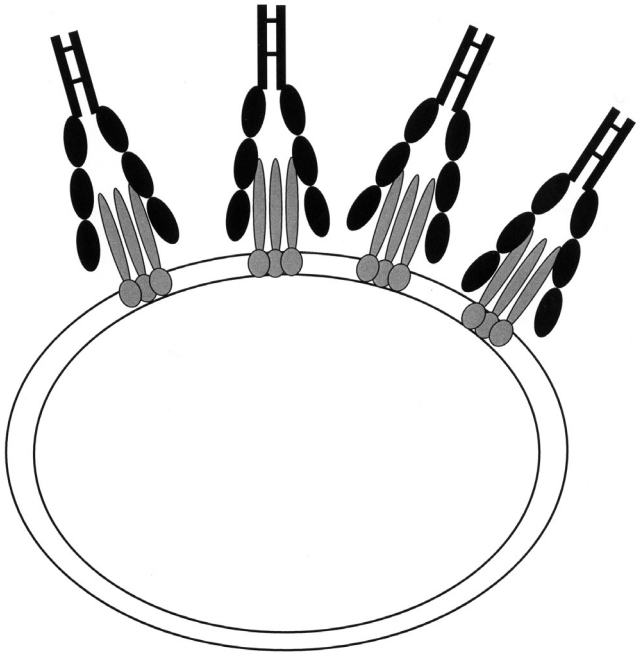

Figure 2. Etanercept binds membrane TNF-α and blocks biologically active TNF-α. Etanercept binds the TNF-α membrane trimer and prevents biological activity. This mechanism of action is relevant to cell-cell contact activation of TNF-α producing cells with TNF-α responding cells. There is no cell death in cells with TNF-α bound to etanercept and the binding is reversible[19].

natural killer cells, but spare cells without membrane surface TNF-α.

Another study confirmed the caspase-dependent cell death mechanism of infliximab[23]. It has become generally accepted that autoimmune T cells isolated from the lamina propria of patients with CD are resistant to cell death and thereby persist as mediators of the disease. The prolonged remission in CD patients treated with infliximab may be due to the elimination of a population of these disease-producing T cells. Certainly, T cells producing membrane TNF-α would fall into this category. In patients with steroid-resistant CD, T cells isolated from intestinal biopsies were obtained before and after 3 consecutive infusions of infliximab[23]. Control biopsies were obtained from subjects with functional diarrhea not receiving infliximab. Using a highly specific assay for measuring apoptosis, T cells from the CD patients were resistant to cell death before infliximab but revealed caspase-dependent cell death after treatment, which was still present 4 weeks after the last infliximab infusion. Importantly, the increase in caspase-dependent cell death was also observed in peripheral blood T cells of these patients after infliximab. Cell death was not due to Fas-mediated mechanisms but rather to activation of caspase-3. The authors concluded that the pro-apoptotic effects of infliximab, which extended far beyond its circulating plasma half-life, account for the long remission in disease activity associated with infliximab in this disease[23]. These and the studies of others clearly implicate T cell death by infliximab[22,24] and are supported by similar findings in circulating monocytes[21].

How does an IgG1 antibody induce caspase-dependent cell death? As shown in Figure 2, cross-linking of membrane TNF-α by IgG1 anti-TNF-α monoclonal antibody forms a lattice configuration. This is a well known property of all complement-fixing bivalent antibodies for surface antigens. It accounts for the lysis of red blood cells in patients with autoimmune hemolytic anemia and the mechanism of cell killing by antibodies to bacteria. Complement activation is required for cell lysis by this mechanism. However, the role of complement activation for caspase-dependent apoptosis is not known. The addition of infliximab to laboratory engineered TNF-α membrane-expressing cells *in vitro* results in cell death by a complement-dependent mechanism[19,20]. However, adding infliximab to naturally occurring T cells from patients with CD results in cell death by activation of caspase-3[22,23]. As shown in Figure 3, one proposed mechanism for activation of caspase-3 by cross-linking of membrane TNF-α on T cells is via reverse signal transduction[25]. It is important to note that in the case of infliximab activation of caspase-3 at 5 μg/ml, T cells are derived *in vivo* and therefore have been activated by the disease process itself[23].

In contrast, etanercept does not lyse cells expressing TNF-α on the membrane[19]. There is no activation of caspase-3 in CD4+ T cells exposed to etanercept[22,26]. Although etanercept is constructed with the complement receptor domains of human IgG1, the hinge region of the fusion of the Fc chain to the p75 extracellular domain of the TNF receptor is missing one of the CH2 groups, and hence the structure is predicted to be more rigid than the Fc of natural antibodies. Although it remains unclear whether the deleted CH2 group explains the difference between etanercept and either infliximab or adalimumab, etanercept does not activate complement and does not lyse cells expressing membrane TNF-α. Soluble TNF receptors lenercept, onercept, and pegsunercept also do not activate complement. Thus, decreases in host defense associated with any method of TNF-α neutralization (infliximab, adalimumab, anti-TNF-α monoclonal antibodies of the IgG4 class, etanercept, lenercept, onercept, and pegsunercept) should be contrasted with the additional role of cell death of membrane TNF-α cells by IgG1 monoclonal antibodies. In the latter case, cell death can be viewed as an "irreversible" event, with the progressive loss of CD4+ and CD8+ T cells. In the case of soluble receptors to TNF or anti-TNF-α of the IgG4 class, the reduction in TNF-α activity by cell-cell contact is "reversible."

Anti-TNF-α monoclonal antibodies affect the numbers of peripheral CD4+ and CD8+ T cells. The vast majority of studies on the effects of anti-TNF-α monoclonal antibodies on T cells are performed in cells from patients with CD. Gastroenterologists readily perform

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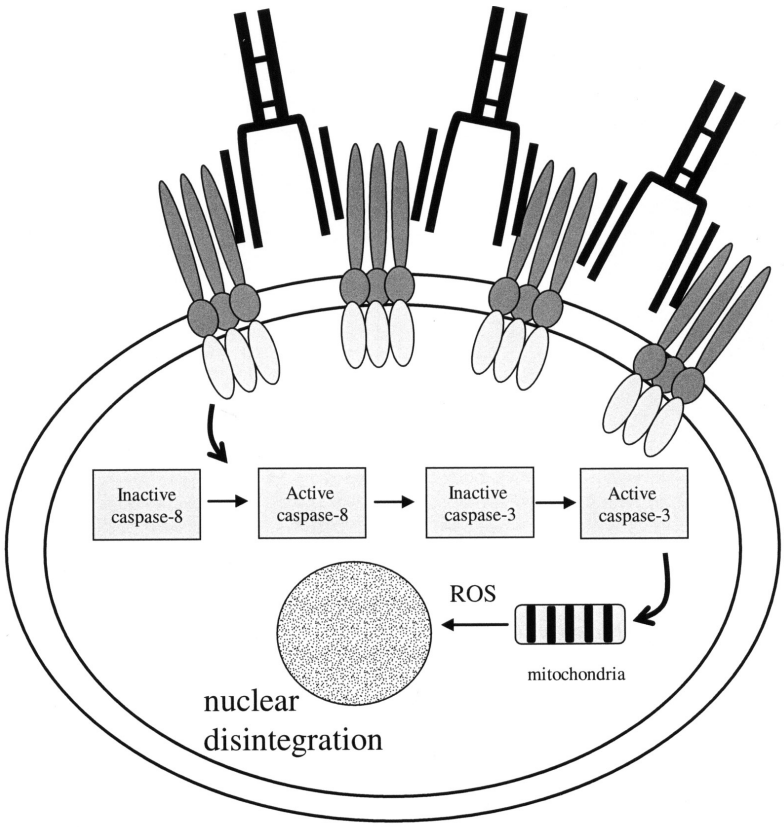

Figure 3. Cross-linking of membrane TNF-α and activation of caspase-3. Anti-TNF-α monoclonal antibodies of the IgG1 class, known to fix complement[19,20], form a lattice with membrane TNF-α. By mechanisms presently unclear, although likely due to reverse signaling[25], a cascade is initiated by which inactive caspase-8 is activated and results in the activation of caspase-3. This has been demonstrated in lamina propria T cells[22,23] and peripheral blood T cells[23] from patients with Crohn's disease. Active caspase-3 results in the release of reactive oxygen species (ROS) from mitochondria, which in turn bring about the disintegration of nuclear DNA, which in turn initiates the process of apoptotic cell death.

colonic biopsies to monitor the severity of disease, making tissues available for clinical and immunological analyses. In contrast, rheumatologists in the United States and Canada rarely perform synovial biopsies to assess disease progression. However, in patients with AS, studies on the percentage of circulating CD4+ and CD8+ T cells have reported significant changes following a course of either infliximab or etanercept. Using specific reagents that detect cells producing TNF-α or IFN-γ, Zou and colleagues removed peripheral blood mononuclear cells (PBMC) before and after therapeutic treatment of AS patients with either infliximab or etanercept compared to placebo-treated patients[5,6]. For infliximab, the assessment is 6 and 12 weeks after 2 consecutive infusions and for etanercept, after 12 weeks of biweekly injections. Although the clinical efficacy for both TNF blockers is similar and statistically significant, the effect of each TNF blocker is different.

PBMC obtained after 6 weeks from both placebo and infliximab-treated patients with AS were stimulated *in vitro* with the combination of phorbol myristate acetate/ionomycin for nonspecific increases in cytokines or the G1 domain of aggrecan as a specific antigen stimulant. The cells were subjected to FACS analysis using T cell surface markers of CD4 and CD8. In addition, staining for intracellular IFN-γ and TNF-α was performed. Endotoxin was used to stimulate monocytes in PBMC preparations and the amount of TNF-α and IL-10 was assessed by specific ELISA. After 12 weeks, AS patients infused with infliximab exhibited a highly significant reduction in the numbers of CD4+ T cells and CD8+ T cells expressing IFN-γ or TNF-α[6]. In fact, the reduction in these cells had already reached statistical significance after 6 weeks, whereas there were no changes in the placebo-treated patients. After 6 weeks, placebo-treated AS patients were infused with infliximab, and a similar significant decrease in IFN-γ and TNF-α expressing CD4+ T cells and CD8+ T cells was observed[6]. Similar results were observed in PBMC stimulated with the specific antigen. Unexpectedly, there was no reduction in the amount

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of TNF-α produced from endotoxin-stimulated PBMC in the patients treated with infliximab.

Using an identical experimental protocol, 10 patients with AS were treated with 25 mg etanercept twice weekly and 10 AS patients received biweekly injections of placebo[5]. In contrast to studies reported above with infliximab, 12 weeks of etanercept resulted in an increase in the percentage of CD4+ and CD8+ T cells expressing TNF-α and IFN-γ[5]. The increases were highly significant, and there were no changes in the placebo-treated group of AS patients. Similar increases were also noted in PBMC stimulated with the specific antigen of aggrecan-derived peptides. The studies provide the first evidence that, similar to patients with CD, patients with a rheumatic disease such as AS also exhibit a reduction in CD4+ T cells when treated with infliximab. The increase in the percentage of CD4+ and CD8+ T cells expressing TNF-α or IFN-γ supports the concept that neutralization of TNF-α per se does not result in a reduction in circulating CD4+ T cells. Loss of CD4+ T cells expressing IFN-γ may contribute to the high incidence of granulomatous disease reported in patients receiving infliximab[12]. Neutralization of peripheral TNF-α does not induce a downregulation of the ability of T cells to produce TNF-α but rather an upregulation, possibly due to a counterregulatory mechanism.

ROLE OF IFN-γ IN INFECTION ASSOCIATED WITH TNF BLOCKERS

One can conclude from large population studies that a well-contained granuloma containing live *M. tuberculosis* is not incompatible with long life as long as the patient's immune system is fully functional. A fully functional immune system includes cytokine production, particularly IFN-γ. Most notably, mutations in any of 5 genes that control production result in severe and life-threatening mycobacterial disease from birth[27]. These include the IFN-γ receptor type I and type II, the p40 chain of IL-12, the IL-12 receptor 1, and intracellular transcription factor STAT 1. These human studies are supported by a large body of animal studies showing failure to contain *M. tuberculosis* infection in mice treated with antibodies to or lacking production of TNF-α, IL-18, IL-12, or IFN-γ. In addition to the disseminated nature of *M. tuberculosis* infection, the organisms are similar to those observed in HIV-1 infected patients with CD4+ T cell counts less than 200/μl. There are, however, notable exceptions: in HIV-1 infection, disseminated infection by *M. avium intracellulare* is common, whereas this is not a common infection in patients receiving TNF blockers. Also, oral candidiasis is not seen in RA treated with TNF blockers, but occurs often in the low CD4+ T cell HIV-1 population. It is important to note that patients treated with anti-TNF-α monoclonal antibodies of the IgG1 class do not exhibit low numbers of circulating CD4+ T cells. In fact, functional characteristics of peripheral T cells in RA patients treated with anti-TNF-α monoclonal antibodies exhibit improved function, a general observation of nearly all antiinflammatory drugs in this disease.

Nevertheless, in both HIV-1 and rheumatoid arthritis populations receiving anti-TNF-α monoclonal antibodies of the IgG1 class, the target cell is a CD4+ T cell. In the case of HIV-1 infection, any cell expressing CD4 on its surface together with CCR5 (the chemokine receptor 5) can be infected and dies an apoptotic death upon viral production. Thus HIV-1 spares the CD8+ T cells while progressively killing off the CD4+ T cell population. In the case of anti-TNF-α monoclonal antibodies of the IgG1 class, any cell expressing TNF-α on its membrane is vulnerable to cell death by either complement-mediated cell lysis or antibody-dependent cytotoxicity[19,20]. Recent studies demonstrate that after infliximab infusion in patients with CD, circulating blood monocytes undergo death by caspase-dependent apoptosis[21]. In peripheral blood, anti-TNF-α monoclonal antibodies of the IgG1 class kill CD4+ T cells by caspase-dependent apoptosis[22]. Again, unlike in HIV-1 infection, these IgG1 antibodies target the surface membrane of any cell expressing TNF-α, including CD4+ T cells, CD8+ T cells, macrophages, and dendritic and natural killer cells, but spare cells without membrane surface TNF-α.

In contrast, etanercept does not lyse cells or activate caspase-3 in cells expressing membrane TNF-α[22]. Although etanercept is constructed with the complement receptor domains of human IgG1, the hinge region of the fusion of the Fc chain to the p75 extracellular domain of the TNF receptor is missing one of the CH2 groups; hence the structure is predicted to be more rigid than the Fc of natural antibodies. Whether this explanation is substantiated or not, it remains that etanercept does not activate complement and does not lyse cells expressing membrane TNF-α. This is also the case with other soluble receptors of TNF (lenercept, onercept, and pegsunercept). Thus, in the context of therapy based on TNF-α neutralization (infliximab, adalimumab, anti-TNF-α monoclonal antibodies of the IgG4 class, etanercept, lenercept, onercept, and pegsunercept), one must consider that any clinical event indicative of decreased host defense against infection may be associated with monoclonal antibodies that fix complement and also result in loss of membrane TNF-α (infliximab and adalimumab).

TESTING FOR RISK OF REACTIVATION OF *M. TUBERCULOSIS*

It is good medical practice to assess the risk of reactivation of tuberculosis before initiating anti-TNF-α therapy. Since routine *M. tuberculosis* testing before initiating TNF blockers was instituted, the number of cases with reactivation *M. tuberculosis* infection has decreased substantially. However, most physicians do not assess anergy when testing for response to purified protein derivative. Indeed, in patients with RA the incidence in anergy has

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been demonstrated. In a study of recall antigens in 48 patients with newly diagnosed RA prior to any treatment with disease modifying antirheumatic drugs, 43.75% of patients exhibited anergy compared to 2% of healthy age and sex-matched controls[28]. The absence of responses to skin testing was not related to clinical or biochemical markers of disease severity. Similar to nearly all anti-inflammatory treatment regimens, depression of cell-mediated immunity can be partially reversed, which was observed in this study using methotrexate but not cyclosporine or hydroxychloroquine[28]. Thus, there are no guarantees when it comes to predictions of reactivation of infection with *M. tuberculosis*.

Although screening for *M. tuberculosis* infection is now routine, and opportunistic infections can be treated with appropriate antimicrobials, one is far less sanguine about an increased risk of non-Hodgkin's lymphoma[29].

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