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The Future Role of Anti–Tumour Necrosis Factor-α Products in the Treatment of Crohn's Disease

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Abstract

Tumour necrosis factor-α (TNFα) is thought to play a central role in the immunopathology of Crohn's disease, particularly since its levels are raised in all types of cells, tissues and secretory fluids of these patients and in animal models of the disease. In addition, TNFα has been found to modulate a number of different processes within the network of inflammatory reactions and therefore has become a target molecule for intervention studies.

In the past few years several compounds have been developed which neutralise or impair the production of TNFα, e.g. monoclonal antibodies [infliximab (cA2), CDP-571], TNF receptor p75-Fc fusion protein, pentoxifylline (oxpentifylline), p65 antisense oligonucleotides and metalloproteinase inhibitors, thereby counteracting the deleterious effects of this proinflammatory cytokine.

At present, successful treatment of active 'refractory' and fistulising Crohn's disease has been reported with anti-TNFα antibodies; more clinical studies are in progress or will be performed with substances that intervene in the activation, production and processing of TNFα. Although important aspects of this type of immune-intervention therapy still need to be elucidated, e.g. long term effects, mechanism(s) of action, identification of responders and nonresponders, etc., it is obvious that the integration of basic and clinical research brings us to a new era of specific cytokine-directed therapy in Crohn's disease.

Crohn's disease is a chronic inflammation of the gastrointestinal tract of unknown cause, often periodically active, and often in remission. The prevalence of the disease in the Western world is about 50 to 100 per 100 000, with an incidence rate of 1 to 10 per 100 000 per year, a figure which has shown a tendency to increase over the past few decades.[1] The disease is primarily localised in the ileum and/or colon, with diarrhoea, abdominal pain and general malaise as the most predominant symptoms. Crohn's disease, however, may also be presented by extraintestinal manifestations such as articular disorders, eye inflammation, dermatological lesions or hepatic disorders. Moreover, the disease is frequently complicated by fistulation, stenosis or abscess formation.[1]

Although the currently available anti-inflammatory medications in Crohn's disease [sulfasalazine, mesalazine (5-aminosalicylic acid), prednisolone] are effective in suppressing disease activity, a considerable number of patients (15 to 40%) either develop or have a more complicated or 'refractory'

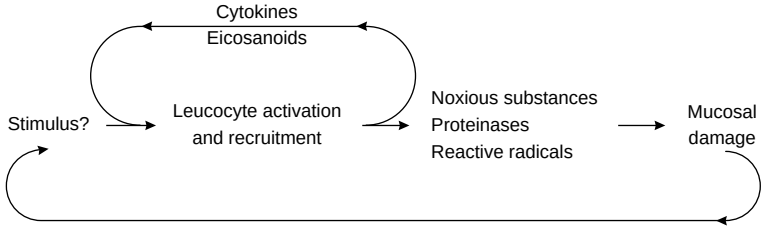

Fig. 1. Sequence of events involved in the inflammatory process of the intestinal mucosa in Crohn's disease.

disease.[1] In these cases, a more aggressive immunosuppressive therapy is the usual choice of treatment (for example azathioprine, 6-mercaptopurine, cyclosporin or methotrexate). Most of these drugs, however, are administered empirically. In the past 10 years compelling evidence has become available that demonstrates the involvement of the immune system in the pathogenesis of inflammatory bowel disease, based on which newer drugs were and still are designed to interfere with immunological systems and mechanisms.[2,3]

When the clinical symptoms of Crohn's disease become apparent it is usually the presentation of the end stage of an immunological/inflammatory response, which matches with that of an antigen-driven process. Large numbers of neutrophils, T cells and macrophages are recruited into the bowel wall leading to a T-helper–1–mediated immune reaction. They are activated by a variety of cytokines and eicosanoids. When activated, they produce more cytokines and inflammatory mediators leading again to more recruitment and activation of cells, thereby constituting a circulatory loop of inflammation.[2]

Several cytokines are present in colonic tissue and/or the circulation of patients with active Crohn's disease, predominantly proinflammatory cytokines such as tumour necrosis factor-α (TNFα) and interleukin (IL)-1, IL-6 and IL-8. However, which of those cytokines play(s) a crucial role in the pathogenesis of the disease has not yet been elucidated. A variety of end-products from the metabolism of arachidonic acid have also been found to be increased, e.g. leukotriene (LT) B_4 and prostaglandin (PG) E_2, in the inflamed intestine. In combination, this cascade of events results in the production of more inflammatory mediators, destructive enzymes and oxygen free radicals that finally lead to tissue injury, as schematically presented in figure 1.

TNFα is thought to play a central role in the orchestration of the mucosal immune response. Produced predominantly in monocytes, macrophages and T cells, TNFα is primarily expressed as a 26kD membrane protein which upon proteolysis is released as a 157 amino acid, 17kD monomeric protein. These monomers can associate into a homotrimer which then constitutes the active form of soluble TNFα.[4,5]

Two receptors for TNFα have been identified (TNF-R p55 and TNF-R p75), which are found on a wide variety of tissue and cell types.[6,7] The TNF-trimer can bind as many as 3 TNF receptors and this cross-linking of receptors initiates signal transduction within the target cell.[8] When TNFα binds to TNF-R p55 a number of actions are initiated, i.e. cytotoxicity, fibroblast proliferation, synthesis of prostaglandins, upregulation of adhesion molecules, nuclear factor binding the B site in the enhancer element of the light chain immunoglobulin κ gene (NF-κB) transcription factor activation, etc.[9] The function of TNF-R p75 is less well understood, but it is thought to concentrate soluble TNFα.[10] In addition, the TNF receptors may be released in a soluble form, upon proteolytic cleavage at the cell surface, and then serve as circulating binding factors which regulate the biological activity of TNFα.

TNFα can induce distinct, overlapping, synergistic or counter-regulatory bioactivities with other cytokines and affects the proliferation, differentiation or function of virtually every cell type. This cytokine modulates a number of different pro-

cesses like suppression of lipoprotein lipase activity, induction of IL-6, activation of endothelial cell adhesion, increase of procoagulant activities and proteolytic enzymes, and stimulation of fibroblast proliferation.[11,12] All these TNFα-mediated biological processes contribute to the initiation, perpetuation and derailment of the mucosal inflammatory process and the subsequent abnormal tissue repair in Crohn's disease, as recently reviewed by van Deventer.[11]

1. Reducing the Activity of Tumour Necrosis Factor-α (TNFα)

There are several ways to reduce the production or effects of TNFα, as reviewed by Eigler et al.[12] and illustrated in figure 2. First, with anti-TNFα antibodies the cytokine can be neutralised. There are 2 antibodies currently available for clinical studies, which bind to released TNFα trimers as well as to membrane-bound TNFα. The first anti-TNFα antibody applied is a chimeric (25% mouse, 75% human) monoclonal antibody immunoglobulin (Ig) G_1 [infliximab (cA2)], genetically engineered using the variable region of the murine monoclonal antibody A2. Another genetically engineered anti-TNFα antibody, CDP-571, is of the IgG_4 subclass and contains about 95% human and 5% murine sequences, which in theory has minimal immunogenicity in humans. Both drugs are administered intravenously. In repeated doses these anti-TNFα antibodies might induce human anti-chimeric antibodies (HACA), which could interfere with their biological efficacy due to immunoneutralisation of the drug.

In order to overcome this adverse effect of anti-TNFα treatment, an alternative approach could be the use of fusion proteins. A recombinant human TNF-R p75-Fc fusion protein, TNFR:Fc, has already been developed. In this protein, DNA encoding the soluble portion of the human TNF-R p75 is linked to DNA encoding the C-terminal halves of the immunoglobulin heavy chain (Fc) portion of the human IgG_1 molecule. The resulting immunoglobulin-like protein is a competitive inhibitor of

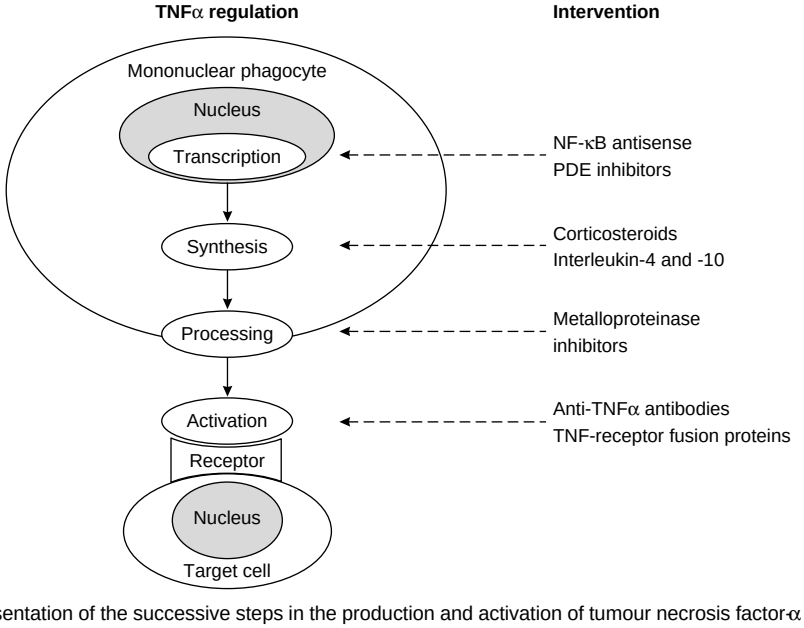

Fig. 2. Schematic presentation of the successive steps in the production and activation of tumour necrosis factor-α (TNFα) and the potential levels and substances of intervention strategies (see section 1). **NF-κB** = nuclear factor binding the B site in the enhancer element of the immunoglobulin κ gene; **PDE** = phosphodiesterase.

TNFα and prevents the binding of the cytokine to its cell surface receptor.[13] Nevertheless, these proteins, which must be administered intravenously, may still be immunogenic, although they are entirely composed of human protein sequences.

Transmembrane TNFα must be released by a TNFα converting enzyme (a metalloproteinase) to exert effect as a soluble cytokine. Metalloproteinase inhibitors have been shown to reduce TNFα production. These TNFα convertase inhibitors reduce the release of TNFα *in vivo* and *in vitro*, but do not alter the expression of membrane-bound TNFα.[11]

Another method of intervention is interference with TNFα production by modulation of the cytokine transcription with phosphodiesterase (PDE) inhibitors, which induce high intracellular cyclic adenosine monophosphate (cAMP) levels and thereby inhibit the production of TNFα.[14,15] For example, pentoxifylline (oxpentifylline), the 1-5-oxohexyl analogue of methylxanthine-theobromine, has already been shown to be effective *in vitro* and applied to treat several disorders.[16]

In trinitrobenzene sulfonic acid (TNBS)-induced colitis and IL-10–deficient mice, the transcription factor NF-κB p65 was found to be highly increased. NF-κB p65 (one of the members of the NF-κB family) has been shown to regulate the transcriptional activity of the promoters of proinflammatory cytokines, including TNFα in macrophages. Local administration of specific p65 antisense oligonucleotides in TNBS-induced colitis was proven to reduce intestinal inflammation and the production of IL-1, IL-6 and TNFα by lipopolysaccharide-stimulated intestinal macrophages in a dose-dependent manner.[17]

2. Therapy in Crohn's Disease with Anti-TNFα

Several studies in different animal models of inflammatory bowel disease and assessment of TNFα production in Crohn's disease patients provided the rationale for immunoneutralisation studies in these patients (see the review by van Deventer[11]). Administration of anti-TNFα infliximab antibody was found to be of substantial benefit to patients with active Crohn's disease. The first preliminary open-label studies in patients with corticosteroid-refractory Crohn's disease revealed a complete clinical remission, a remarkable improvement of endoscopic features and substantial improvement of biochemical parameters in a large majority of the patients.[10]

To investigate the safety, tolerance and response to this infliximab antibody, an open-label, multicenter, dose-escalating study was performed in 20 patients with active Crohn's disease who had been taking prednisone ≥15 mg/day for at least 1 month. They were treated with a single intravenous injection of either 1, 5, 10 or 20 mg/kg infliximab, 5 patients receiving each of these doses, and followed for 12 weeks. 90% (18 of 20) showed a clinical response during the first 4 weeks, i.e. a drop of at least 70 points in the Crohn's Disease Activity Index (CDAI), without a clear dose-effect relationship. Clinical remission (CDAI < 150 points) was achieved in an average of 40% (20 to 60%) of the patients, again with no major differences among the doses. After 12 weeks, in the lowest dose group only 1 of 5 patients included still had a response, whereas in the other groups 2 to 4 patients were still experiencing clinical benefit. The severity of endoscopic lesions was substantially reduced in the 3 highest dose groups. One patient had a haemolytic anaemia after 2 to 3 months and in 1 patient a partial ileum resection had to be performed because of intestinal obstruction.[18] Histological improvement was seen in biopsies of 13 of the 15 patients treated with the highest doses. The histological score (range 0 to 16) dropped from 6.7 to 3.0 in the ileum and from 7.6 to 3.0 in the colon.[19]

In the first controlled trial,[18] a total of 108 patients with active Crohn's disease received either 0, 5, 10 or 20 mg/kg anti-TNFα infliximab antibody intravenously. Clinical response (a drop in the CDAI by ≥70 points) was demonstrated in 17, 81, 50 and 64%, respectively, of the patients in the different dose-groups. Combined response among all the infliximab treatment groups was 65% ($p < 0.001$). The clinical response was achieved early,

i.e. 61% of treated patients had a clinical response at the second week versus 17% in the placebo group. In addition, at 4 weeks 33% of the infliximab-treated patients were in remission, compared with only 4% of the placebo group ($p < 0.005$), which was accompanied by a significant endoscopic improvement of ileocolonic lesions.[20] Even as early as after 2 weeks the CDAI had decreased by a mean of 100 points (all infliximab groups combined) compared with 16 points in the placebo group ($p < 0.001$). A few serious adverse effects, such as infusion-related reactions, abscess formation and intestinal obstruction, have been detected.[21]

This study was continued with those patients who showed a good clinical response at week 8. The patients were subsequently given 4 doses of 10 mg/kg anti-TNFα or placebo at 8-week intervals. 73 of the 108 patients enrolled in this study. The patients receiving retreatment with infliximab had benefit until 44 weeks after the initial (active disease) treatment. In the placebo group, progressively fewer patients showed a response. At week 44 a better clinical response, though not statistically significant, and clinical remission ($p < 0.05$) was observed in the treatment group.[22]

In a recently published study[23] of infliximab treatment in Crohn's disease, 94 patients with longstanding single or multiple draining fistulae (abdominal or perianal) were given 3 infusions of placebo or infliximab 5 or 10 mg/kg at weeks 0, 2 and 6. Closure of at least 50% of the fistulae was obtained in 67.7 and 56.3% of the patients in the 5 and 10 mg/kg groups, respectively, as opposed to 25.8% of the patients in the placebo group. The median time to response was 14 days in the infliximab group versus 42 days in the placebo group. Thus, administration of anti-TNFα antibodies caused a rapid closure of more than 50% of the draining fistulae in a majority of the Crohn's disease patients.

CDP-571, the other anti-TNFα antibody for clinical use, has been investigated in a controlled pilot trial in 31 patients with active Crohn's disease.[24] 21 patients received the active drug intravenously (5 mg/kg). The CDAI in these CDP-571-treated patients fell significantly within 2 weeks, while no effect was found in the placebo group. In 6 patients treated with CDP-571 remission was achieved. Although these results are very similar to those of the larger infliximab trial, the sustained effects over a longer period, i.e. 4 to 8 weeks, observed with infliximab were not found with CDP-571. After a single injection human antibodies to CDP-571 were detected in 35% of the patients and only mild adverse reactions were reported.[24] Other larger studies with this neutralising antibody are currently under way.

The mechanism(s) by which the genetically engineered anti-TNFα antibodies exert their activity has not yet been fully elucidated. Neutralisation of circulating and cell surface–bound TNFα, thereby intervening in the activated immunological cascades of cytokine production and immune cell stimulation, seems to be the primary function. Direct cytotoxicity (depletion) by antibody- and complement-mediated lysis of TNFα-bearing cells seems to be less important since CDP-571 IgG_4 does not fix complement, as opposed to the infliximab IgG_1. Further studies in the near future will certainly reveal more routes by which these antibodies act.

Modulation of cytokine production by intervention at the transcription level was investigated *in vitro* with peripheral blood mononuclear cells and intestinal biopsies in patients with active Crohn's disease and ulcerative colitis.[15] Pentoxifylline was able to inhibit the release of TNFα by up to 50% in the cells and tissues of these patients. Therefore, it seems to be potentially an interesting drug for patients with inflammatory bowel disease. However, in a preliminary open-label study with 16 corticosteroid-dependent Crohn's disease patients, pentoxifylline 400mg 4 times daily for 4 weeks did not improve the CDAI or endoscopic score, although *in vitro* TNFα production by peripheral blood monocytes was considerably impaired.[25]

The efficacy of NF-κB p65 antisense oligonucleotides to affect cytokine production was assessed *in vitro* using intestinal macrophages of 18 Crohn's disease patients. A significant down-regulation of

TNFα, IL-1 and IL-6 production was observed which was more pronounced with the p65 antisense oligonucleotide than with corticosteroids or mesalazine.[17] The recent preliminary observation of an effective treatment of corticosteroid-dependent Crohn's disease with antisense oligonucleotides to intracellular adhesion molecule–1, which is also under the control of TNFα, indicates the feasibility of using these substances as systemic drugs.[26]

3. Future Therapy with Anti-TNFα in Crohn's Disease

For the future, it is expected that a shift will take place in the management of patients with refractory Crohn's disease. The success of the first trials with anti-TNFα antibodies has encouraged the design of newer studies. Although not all modes of therapy with these antibodies have been extensively studied to date in Crohn's disease, it is evident that neutralising anti-TNFα antibodies are good candidates to become successful drugs. Further large-scale studies of such agents, in combination with other immunomodulatory drugs, but also as primary therapy or to maintain steroid-induced remission in Crohn's disease, are to be anticipated. However, at the same time more information must be obtained on optimal dosage, long term efficacy and adverse effects, (genetic) identification of (non)responders, underlying activity mechanisms, etc.

The recently performed controlled study in rheumatoid arthritis in which 180 patients were treated with subcutaneous injections of the TNFR:Fc compound led to the conclusion that it was well tolerated and associated with improvement in the inflammatory symptoms of these patients. In addition, no antibodies to TNFR:Fc were formed and there were no dose-limiting toxic adverse effects.[27] It might be expected that similar studies in Crohn's disease based on these encouraging results will be performed in the near future, although negative results have been reported in the treatment of septic shock with this fusion protein.[28]

Clinical studies in Crohn's disease with drugs which intervene in the production and processing of TNFα, e.g. with antisense oligonucleotides, new and selective PDE inhibitors, metalloproteinase inhibitors or anti-inflammatory cytokines like IL-10, have not yet been reported in detail (only some preliminary or anecdotal data are available),[2,3,11] but future clinical studies are being designed. For these strategies, however, important aspects including route of administration, adverse effects, tolerability and mode of action should also be analysed further in animal models and in patients. Nonetheless, an exciting new array of immune-intervention strategies for Crohn's disease is emerging.

References

1. Targan SR, Shanahan F, editors. Inflammatory bowel disease: from bench to bedside. Baltimore: Williams & Wilkins, 1994
2. Elson CO. The basis of current and future therapy for inflammatory bowel disease. Am J Med 1996; 100: 656-62
3. Sands BE. Biologic therapy for inflammatory bowel disease: clinical review. Inflamm Bowel Dis 1997; 3: 95-113
4. Aggarwal BB, Kohr WJ, Hass PE, et al. Human tumor necrosis factor: production, purification and characterization. J Biol Chem 1985; 260: 2345-54
5. Smith RA, Baglioni C. The active form of tumor necrosis factor is a trimer. J Biol Chem 1987; 262: 6951-4
6. Scheurich P, Thoma B, Ucer U, et al. Immunoregulatory activity of recombinant human tumor necrosis factor (TNF)-α: induction of TNF receptors on human T-cells and TNF-α mediated enhancement of T-cell responses. J Immunol 1987; 138: 1786-90
7. Hohmann HP, Remy R, Brockhaus M, et al. Two different cell types have different major receptors for human tumor necrosis factor (TNFα). J Biol Chem 1989; 264: 14927-34
8. Pennica D, Kohr WJ, Fendly BM, et al. Characterization of a recombinant extracellular domain of the type 1 tumor necrosis factor receptor: evidence for tumor necrosis factor-α-induced receptor aggregation. Biochemistry 1992; 31: 1134-41
9. Mackay F, Loetscher H, Stueber D, et al. Tumor necrosis factor α (TNF-α)-induced cell adhesion to human endothelial cells is under dominant control of one TNF receptor type, TNF-R55. J Exp Med 1993; 177: 1277-86
10. Tartaglia LA, Pennica D, Goeddel DV. Ligand passing: the 75-kDa tumor necrosis factor (TNF) receptor recruits TNF for signaling by the 55-kDa TNF receptor. J Biol Chem 1993; 268: 18542-8
11. van Deventer SJH. Tumor necrosis factor and Crohn's disease. Gut 1997; 40: 443-8
12. Eigler A, Sinha B, Hartmann G, et al. Taming TNF: strategies to restrain this proinflammatory cytokine. Immunol Today 1997; 18: 487-92
13. Moreland LW, Margolies G, Heck Jr LW, et al. Recombinant soluble tumor necrosis factor receptor (p80) fusion protein: toxicity and dose finding trial in refractory rheumatoid arthritis. J Rheumatol 1996; 23: 1849-55
14. Bessler H, Gilgal R, Djaldetti M, et al. Effect of pentoxifylline on the phagocytic activity, cAMP levels, and superoxide anion production by monocytes and polymorphonuclear cells. J Leukocyte Biol 1986; 40: 747-54

15. Reimund JM, Dumont S, Muller CD, et al. *In vitro* effects of oxpentifylline on inflammatory cytokine release in patients with inflammatory bowel disease. Gut 1997; 40: 475-80
16. Teixeira MM, Gristwood RW, Cooper N, et al. Phosphodiesterase (PDE)$_4$ inhibitors: anti-inflammatory drugs of the future? Trends Pharmacol Sci 1997; 18: 164-70
17. Neurath MF, Pettersson S, Meyer zum Büschenfelde K-H, et al. Local administration of antisense phosphorothioate oligonucleotides to the p65 subunit of NF-κB abrogates established experimental colitis in mice. Nature Med 1996; 2: 998-1004
18. McCabe RP, Woody J, van Deventer S, et al. A multicenter trial of cA2 anti-TNF chimeric monoclonal antibody in patients with active Crohn's disease [abstract]. Gastroenterology 1996; 110: A962
19. Baert F, Peeters M, D'Haens G, et al. Impressive histologic improvement after TNF antibody (cA2) therapy in active Crohn's disease [abstract]. Gut 1996; 39 Suppl. 1: A17 (T68)
20. D'Haens GR, van Devener SJH, van Hogezand RA, et al. Anti-TNFα monoclonal antibody (cA2) produces endoscopic healing in patients with treatment-resistant active Crohn's disease [abstract]. Gastroenterology 1998. In press
21. Targan SR, Hanauer SB, van Deventer SJH, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor α for Crohn's disease. N Engl J Med 1997; 337: 1029-35
22. Rutgeerts P, D'Haens G, van Deventer SJH, et al. Retreatment with anti-TNFα chimeric antibody (cA2) effectively maintains cA2-induced remission in Crohn's disease [abstract]. Gastroenterology 1997; 112: A1078
23. van Deventer SJH, van Hogezand RA, Present D, et al. Controlled study of anti-TNFα treatment for enterocutaneous fistulae complicating Crohn's disease [abstract no. A01.11]. United European Gastroenterology Week 1997 Oct 18-23; Birmingham
24. Stack WA, Mann SD, Roy AJ, et al. Randomized controlled trial of CDP571 antibody to tumour necrosis factor-α in Crohn's disease. Lancet 1997; 349: 521-4
25. Bauditz J, Haemling J, Ortner M, et al. Treatment with tumour necrosis factor inhibitor oxpentifylline does not improve corticosteroid dependent chronic active Crohn's disease. Gut 1997; 40: 470-4
26. Yacyshyn B, Woloschuk B, Yacyshyn MB, et al. Efficacy and safety of ISIS 2302 (ICAM-1 antisense oligonucleotide) treatment of steroid-dependent Crohn's disease [abstract]. Gastroenterology 1997; 112: A1123
27. Moreland LW, Baumgartner SW, Schiff M, et al. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. N Engl J Med 1997; 337: 141-7
28. Fisher Jr CJ, Agosti JM, Opal SM, et al. Treatment of septic shock with the tumor necrosis factor receptor: Fc fusion protein. N Engl J Med 1996; 334: 1697-702

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