Tumor Necrosis Factor as a Pharmacological Target

Pietro Ghezzi[*,1,2] *and Anthony Cerami*[2]

Abstract

As indicated by its name, tumor necrosis factor (TNF), cloned in 1985, was originally described as a macrophage-derived endogenous mediator that can induce hemorrhagic necrosis of solid tumors and kill some tumor cell lines in vitro. Unfortunately, its promising use as an anticancer agent was biased by its toxicity, which was clear soon from the first clinical trials with TNF in cancer. Almost at the same time TNF was being developed as an anticancer drug, it became clear that TNF was identical to a mediator responsible for cachexia associated with sepsis, which was termed cachectin. This research led to the finding that TNF is, in fact, the main lethal mediator of sepsis and to the publication of a huge number of articles showing that TNF inhibits the toxic effects of bacterial endotoxins, which are now described as systemic inflammatory response. Although the clinical trials with anti-TNF in sepsis have not been successful thus far, undoubtedly as a result of the complexity of this clinical setting, these studies ultimately led to the identification of TNF as a key inflammatory mediator and to the development of anti-TNF molecules (soluble receptors and antibodies) for important diseases including rheumatoid arthritis and Crohn's disease. On the other side, the mechanisms by which TNF and related molecules induce cell death have been studied in depth, and their knowledge might, in the future, suggest means of improve the therapeutic index of TNF in cancer.

Index Entries: Tumor necrosis factor; cachectin; sepsis; inflammation.

1. History of TNF

1.1. The Era of Soluble Mediators and the Magic Bullets Against Cancer

The 1970s and 1980s were the golden age of cytokines when the biochemical nature of several soluble mediators was clarified. Cellular immunologists then identified macrophage-derived mediators that activate lymphocytes (LAF, lymphocyte-activating factor) and lymphocyte-derived mediators that activate macrophages (MAF, macrophage-activating factor). These molecules are in the class of mediators defined as growth factors, which include hematopoietic growth factors (that now retain those names: G-CSF, GM-CSF, EPO), and interferons (described as antiviral factors in the late 1950s). One particularly active field was that of the research of soluble mediators that could kill tumor cells or boost anticancer defense. Along this line, earlier studies focused on a lymphocyte-derived cytotoxin termed lymphotoxin (LT), and led to the discovery of a serum factor capable of inducing hemorrhagic necrosis of tumors in vivo and killing of tumor cells in vitro. This factor was termed tumor necrosis factor (TNF) and shown to be mainly a macrophage product, as opposed to LT. In 1985 several groups reported the cloning of human and mouse TNF and the ability of recombinant TNF to induce hemorrhagic necrosis of tumors in mice. It would have not been easy, 15 yr ago, to predict that the main clinical application of the discovery and characterization of TNF would have consisted in the administration of anti-TNF molecules for the therapy of rheuma-

*Author to whom all correspondence and reprint requests should be addressed. Mario Negri Institute, via Eritrea 62, Milan 20157, Italy; [2]Kenneth S. Warren Institute, Ossining, NY10562.. E-mail: ghezzi@marionegri.it.

toid arthritis and Crohn's disease. In fact, TNF turned out to be a key pathogenic mediator with pleiotrophic activities, and its history and road, from immunity to inflammation, very similar to that of interleukin (IL)-1. Also unexpected is the fact that the characterization of the inflammatory action of TNF stemmed from studies on models of sepsis.

1.2. From Cancer and Immunity to Endotoxic Shock and Septic Shock

Studies on the molecular basis of cachexia associated with sepsis led to the finding that macrophages activated with endotoxin, used to reproduce settings of septic shock, release a factor that is cachectogenic in vivo and inhibits lipogenesis in cultured adipocytes. We termed this factor cachectin and, when we purified it, found that it was identical to TNF.

These earlier studies pointed out a pathogenic role for TNF in sepsis and inflammation, confirmed by earlier clinical trials with recombinant TNF in cancer patients showing toxicity in phase I and II studies.

The first studies of neutralization of endogenous TNF have shown that this cytokine is a lethal mediator associated with toxicity of endotoxin *(1)* and septic shock induced by live bacteria *(2)*. These studies have pointed at the possible use of anti-TNF antibodies in the therapy of septic shock. However, the clinical trials conducted so far have not indicated a clear efficacy of anti-TNF in septic shock.

After a period of great enthusiasm, during which septic shock was considered the prototypic cytokine-mediated disease, the complexity of this pathological condition, which is associated with other diseases such as cancer, trauma, or burn injury scared most of the big pharmaceutical companies from pursuing this line. Some attempts have been made toward a narrower definition of the component of septic shock, including acute respiratory distress syndrome (ARDS) and multiple organ failure (MOF), in which cytokines play an important role. In 1992 the American College of Chest Physicians and the Society of Critical Care Medicine Consensus Conference introduced the term systemic inflammatory response syndrome (SIRS) *(3)*. Despite these difficulties, the scientists working in the field of cytokine and inflammation have continued using the models of lipopolysaccharide (LPS) toxicity as a means of inducing a systemic inflammatory response (to stick to the above-mentioned definition).

1.3. Lipopolysaccharide Toxicity as a Model of Inflammation (From Endotoxic Shock to Arthritis)

The studies on the role of TNF and IL-1 in septic shock have stimulated a large number of studies on these cytokines in several models of inflammation. These included demonstration of inflammatory effects of TNF administration in various models in vivo and in vitro, as well as reports of increased TNF production in patients or animal models of diseases including rheumatic diseases.

Soon after their characterization, cytokines were suggested to be involved in arthritis. The same year TNF was cloned, Dayer et al. *(4)* and Saklatvala *(5)* reported that TNF was able to induce prostaglandin and collagenase production by synovial cells and stimulate resorption in cartilage, and suggested its pathogenic role in rheumatoid arthritis.

The development of anti-TNF antibodies was the first strategy to inhibit TNF (discussed previously). Anti-TNF antibodies, then soluble TNF receptors (discussed later), and, more recently, IL-1ra, are now approved drugs for the therapy of rheumatoid arthritis or Crohn's disease.

Retrospectively, one can say that the models of LPS toxicity in vivo have been predictive of an anti-inflammatory action in diseases where inflammation is induced in the absence of sepsis.

2. Endogenous TNF Inhibitors and Inhibitory Pathways

It is impossible to cite all the molecules that have been shown to inhibit TNF production or action. From the perspective of basic research and immunopathology, the endogenous inhibitors of TNF are particularly interesting, but also indicates inhibitors of TNF production of possible pharmacological interest. This was particularly evident

for one inhibitor, soluble TNF receptor (sTNFR), that is now a widely used anti-TNF drug.

2.1. Soluble Receptors

As early as 1988 Seckinger et al. reported the existence of a TNF inhibitor in human urine *(6)*, soon identified as a soluble form of the TNF receptor *(7,8)*. As a consequence of these studies, administration of recombinant soluble TNF receptor, both the native molecule and the engineered Fc fusion protein, developed to increase the plasma half-life, were tested in models of disease and are at the basis of the current use of these molecules in patients with inflammatory diseases.

2.2. Glucocorticoids and the Neuroendocrine System

Glucocorticoids have been the first reported inhibitors of TNF production *(9)*. Their action is mediated by the glucocorticoid receptor and reversed by GC receptor antagonist mifepristone *(10)*. It should be noted that other steroids, namely neurosteroids, inhibit TNF production by a glucocorticoid receptor-independent mechanism *(11)*.

Endogenous glucocorticoids probably represent the most important feedback system to limit TNF production, as demonstrated by the augmentation of TNF-mediated endotoxic shock in adrenalectomized mice, known for a long time *(12,13)*, and by similar results obtained with mifepristone *(14,15)*. It is now recognized that TNF increases serum glucocorticoid levels through the activation of the hypothalamus pituitary adrenal axis *(16)*.

2.3. Prostaglandins and Cyclic AMP

The inhibitory effect of the phosphodiesterase inhibitors on TNF production has been described soon after the discovery of TNF. In particular, pentoxifylline and rolipram have been widely used to inhibit TNF production in several animal models. Their inhibition is mediated by the increase in intracellular cyclic AMP (cAMP). Likewise, other agents that augment intracellular cAMP (particularly prostaglandin E_2) inhibits TNF production.

In fact, prostaglandin E_2 is another very important feedback inhibitor of TNF (and other cytokines) production because inhibitors of prostaglandin synthesis (cyclooxygenase inhibitors also known as nonsteroidal anti-inflammatory drugs) augment cytokine production in most models ranging from in vitro systems *(17)* to human volunteers injected with LPS and in vivo *(18,19)*. This effect demonstrates that prostaglandin E_2 endogenously produced during inflammation effectively switches off TNF synthesis.

Based on these findings, several clinical trials have been initiated using phosphodiesterase inhibitors to augment intracellular cAMP, such as rolipram or pentoxifylline. Clearly, this approach is not specific for TNF.

2.4. Anti-inflammatory Cytokines (IL-10, IL-13)

Interleukin-10 and IL-4 are the prototypic "anti-inflammatory cytokines" and inhibit TNF production in vitro and in vivo *(20–22)*. This effect was later demonstrated with IL-13 *(23)* and other cytokines of the so-called IL-10 family *(24)*. These anti-inflammatory cytokines (according to PubMed this term was actually used first for IL-4; *see* **ref.** *22*) are being investigated as possible anti-inflammatory drugs.

2.5. The Cholinergic Anti-inflammatory Pathway

Studies by Borovikova et al. *(25)* and Tracey *(26)* using vagotomized animals or electrical stimulation of the vagus nerve have shown that efferent activity in the nerve inhibits TNF production and has anti-inflammatory actions.

This pathway has been termed the cholinergic anti-inflammatory pathway, as inhibition of TNF synthesis is mediated by acetylcholine acting on nicotinic bungarotoxin-sensitive acetylcholine receptors on macrophages. This finding provides a new means of inhibiting TNF production by electrical or chemical methods.

3. Other TNF Inhibitors

It is of no surprise that the effectiveness of recombinant proteins acting as TNF inhibitors has prompted the research of small molecular weight drugs to act as inhibitors of TNF production and that might be administered orally.

Several classes of drugs have been reported to act in this context, but none, as far as we know, are specific for TNF. In particular, no small TNF receptor antagonists have been described to the best of our knowledge. Following is a (partial) list of drugs, or classes of drugs, that reportedly inhibit either TNF production or TNF action and that have been shown to be efficacious in animal models of inflammation.

3.1. Inhibitors of Nuclear Factor-κB

Nuclear factor (NF)-κB is a transcription factor implicated in the expression of several inflammatory genes including TNF, and it is regarded as a major pharmacological target for anti-inflammatory drugs. A long list of well-known molecules were reported to inhibit NF-κB, including antioxidants *(27)*, glucocorticoids *(28)*, aspirin, and salicylates *(29)*.

3.2. Metalloprotease Inhibitors

The TNF-α is synthetized as a membrane-anchored translation product. It is processed to mature TNF, which is then released, by TNF-α-converting enzyme (TACE), a membrane protease in the class of the ADAM proteases (that contain *a d*isintegrin and *a m*etalloprotease domain). Inhibitors of TACE are thus potential anti-TNF molecules and some have been shown active in various animal models of TNF-mediated pathologies *(30)*.

3.3. Thalidomide

This old drug was shown to inhibit TNF production in 1991 *(31,32)*. It is now considered for rheumatoid arthritis and Crohn's disease, and the search for analogs without its teratogenic properties is actively pursued.

3.4. p38 Mitogen-Activated Protein Kinase (MAPK) Inhibitors

Unlike the cAMP pathways, the p38MAPK pathway has been identified only after the discovery of TNF and IL-1. This kinase was originally described by Lee et al. *(33)*. By studying the mechanism of action of a new series of compounds acting as cytokine synthesis inhibitors they identified p38 MAPK by photoaffinity labeling. This kinase was soon identified as a key step in the pathway leading to cytokine production and action.

Another compound that was described as an inhibitor of TNF production and was then found to act probably by inhibiting p38MAPK is the guanylhydrazone CNI1493 *(34,35)*. CNI1493 showed promising activity in patients with Crohn's disease *(36)*.

4. Back to Immunity and Host Defense

Although this short historic overview was focused on the pro-inflammatory actions of TNF and anti-TNF strategies, it is important to remember that TNF is also a key mediator in host defense and innate immunity.

These are probably exemplified by the increased incidence of infections in patients with arthritis given anti-TNF molecules, an observation that is now incorporated in the prescription information for these drugs, advising to avoid use in patients with underlying sepsis (see prescription information). Although this finding was not totally unexpected (other drugs used for the therapy of rheumatoid arthritis, methotrexate and glucocorticoids, are, by definition, immunosuppressive drugs), it reinforces the animal data showing that TNF is a key molecule in the innate immunity to infection.

Nevertheless, the successful therapeutic applications with anti-TNF molecules for a variety of diseases stresses the deleterious effects of its overproduction.

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