Therapeutic potential of TNF-α inhibitors old and new

J. Blake Marriott, Michael Westby and Angus G. Dalgleish

Tumour necrosis factor-alpha (TNF-α) is a proinflammatory cytokine that has been implicated in the pathogenesis of a spectrum of medical disorders. Mononuclear cells that produce excessive amounts of TNF-α at sites of inflammation are primary targets for therapeutic intervention; immunosuppressive compounds with unpleasant side effects are now being replaced by specific anti-TNF-α formulations. In this review we examine a number of compounds that are currently being evaluated for efficacy both *in vitro* and in ongoing clinical studies. The variety of compounds available reflects the increase in our understanding of the mechanisms involved in the production and action of TNF-α.

Tumour necrosis factor-alpha (TNF-α) is produced by many cell types, including monocytes and macrophages, T and B lymphocytes, neutrophils, mast cells, tumour cells and fibroblasts. It is a pleiotropic molecule produced in response to a variety of stimuli and can exert effects on most cell types. TNF-α is a key regulator of other proinflammatory cytokines, including interleukin 1β (IL-1β), IL-6 and IL-8. During normal host defence, low levels of serum TNF-α confer protection against infectious agents, tumours and tissue damage, and have an important role in the development of the humoral immune response in mice[1]. On the other hand, overproduction of TNF-α can lead to autoimmunity, malignancy or inflammatory and immunopathological disease[2–4] (Box 1). The systemic overproduction of TNF-α during infection with Gram-negative bacteria, for example, induces widespread tissue damage and in severe cases can lead to death from circulatory shock. The importance of TNF-α in conferring both pathogenesis and protection is demonstrated in a mouse model of lipopolysaccharide (LPS)-induced shock in which deletion of the TNF receptor (55 kDa) gene is totally protective against LPS-induced elevated TNF-α production[5,6]. However, the same mice have a weakened host defence system, as shown by their high susceptibility to infection by the intracellular bacterium *Listeria monocytogenes*.

Human TNF-α is synthesized as a membrane-bound polypeptide precursor (26 kDa), which is processed to a soluble form (17 kDa) by proteolytic cleavage. This soluble form trimerizes to give the mature bioactive homotrimeric

Box 1. Tumour necrosis factor-alpha (TNF-α) production in health and disease

Beneficial effects	Harmful effects
Host defence:	Immunopathology
Antiviral	Allograft rejection
Antitumour	Autoimmunity
Antibacterial	Septic shock
Antiparasite	Malignancy
	Cachexia
Inflammation	

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polypeptide (52 kDa) TNF-α molecule. The membrane-bound TNF-α precursor can mediate typical soluble TNF-α responses such as cytotoxicity[7] and B cell activation[8]. TNF-α exerts its multiple biological effects via interaction with two structurally and functionally distinct high-affinity receptors: TNFR1 (55–60 kDa) is expressed on all cells apart from unstimulated T cells and erythrocytes, and TNFR2 (75–80 kDa) is expressed on haemopoietic and endothelial cells. Binding of TNF-α to these receptors results in the activation of several ill-defined signal transduction pathways, leading to expression of a large number of cellular genes[2,9,10] (Figure 1). Receptors can be shed from the cell surface; this provides a possible mechanism for regulating the bioavailability of TNF-α because soluble receptors competitively inhibit interaction of TNF-α with membrane-bound receptor[11] and stabilize the TNF-α molecule[12].

Overproduction of TNF-α is associated with a wide range of pathological conditions (Box 2). This has led to much recent effort in finding ways to downregulate its production or inhibit its effects. Many drugs that are commonly used as

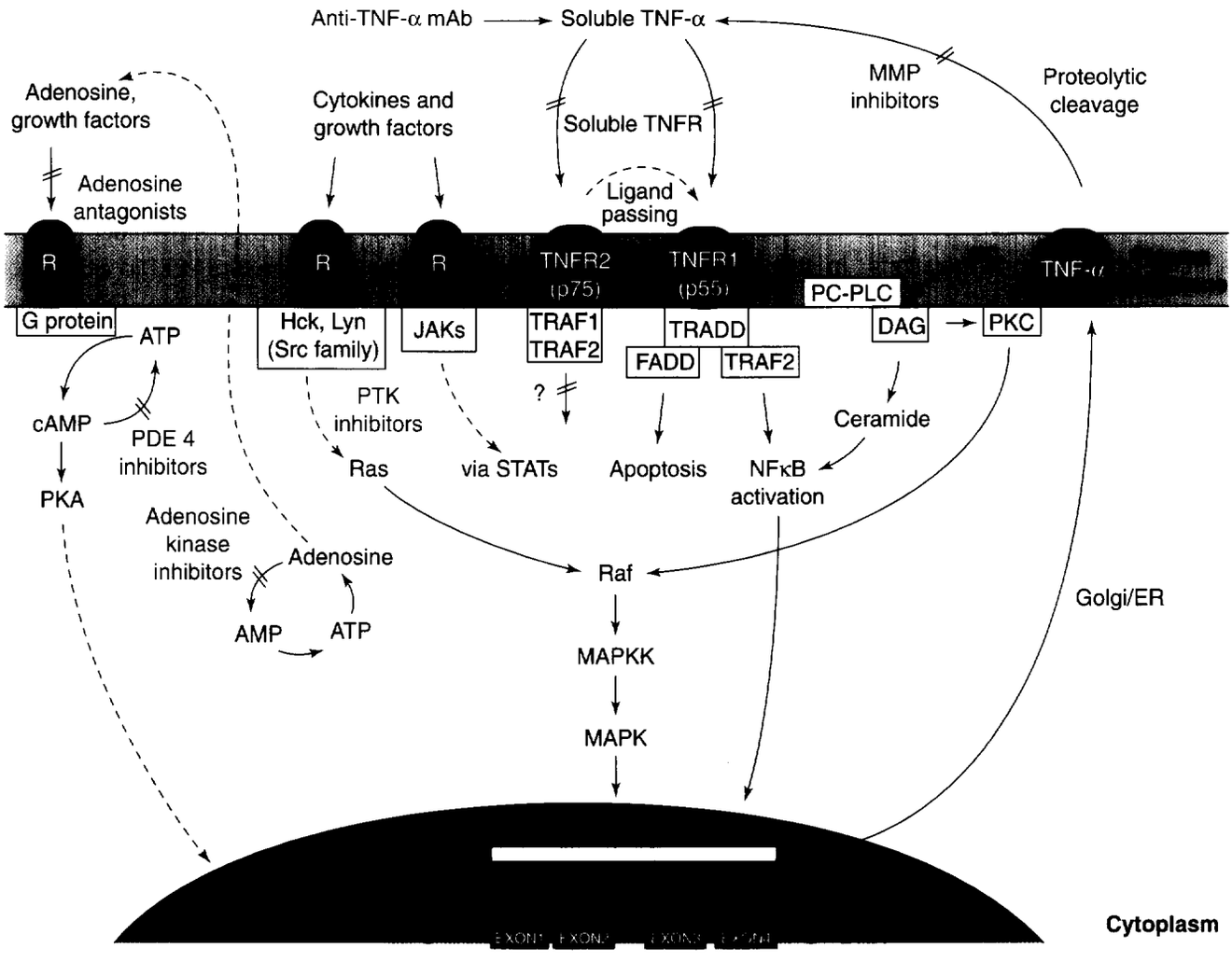


Figure 1. *Points of intervention of tumour necrosis factor-alpha (TNF-α) inhibitors discussed in the text. cAMP, adenosine 3′,5′-cyclic monophosphate; DAG, diacylglycerol; FADD, Fas-associated death domain protein; JAKs, Janus kinases; mAb, monoclonal antibody; MAPK, mitogen-activated protein kinase; MAPKK, MAPK + kinase; MMP, matrix metalloproteinase inhibitor; PC, phosphatidylcholine; PC-PLC, phosphatidylcholine-specific phospholipase C; PDE 4, phosphodiesterase type 4; PKA/C, protein kinase A/C; PTK, protein tyrosine kinase; R, various receptors; STAT, signal transducer and activator of transcription; TNFR, TNF receptor; TRADD, TNFR1 associated death domain protein; TRAF, TNF-receptor-associated factor.*

Box 2. Conditions associated with overproduction of tumour necrosis factor-alpha (TNF-α)

Autoimmunity
Crohn's disease
Insulin-dependent diabetes mellitus
Multiple sclerosis
Psoriasis
Rheumatoid arthritis
Systemic lupus erythematosus

Infectious agents
Cerebral malaria
Hepatitis C
HIV infection
Septic shock
Tuberculosis

Tumours
Angiogenesis
Lymphoma
Ovarian cancer

Other
Aphthous ulceration
Asthma
Behçet's syndrome
Cachexia
Cardiac disease
Erythema nodosum leprosum
Glomerulonephritis
Graft-versus-host disease (GVHD)
Jarisch–Herxheimer reaction
Pancreatitis
Parkinson's disease

immunosuppressants, such as cyclosporin A and dexamethasone, show TNF-α inhibitory properties, although their effects are broad and associated with considerable toxicity. However, as we understand more about the mechanisms involved in TNF-α production and its action on cells, more specific strategies are emerging. This is leading both to the design of new drugs and the modification of existing ones to create less toxic, more potent and potentially more specific alternatives.

Anti-TNF-α antibodies

Anti-TNF-α antibodies have been used to treat conditions associated with elevated TNF-α. Initially, this was demonstrated by the use of passive immunization to prevent lethal endotoxaemia in mice[13]. However, mixed results were obtained during early Phase I/II trials in humans when murine anti-TNF-α monoclonal antibody (mAb) was used to treat patients with conditions in which TNF-α was associated with pathogenesis. For example, 14 patients with septic shock who did not respond to conventional therapy were given CB0006, a murine IgG1-κ mAb, in a Phase I study, which led to increased mean arterial pressure and the survival of three of the patients at day 28 (Ref. 14). A placebo-controlled study of CB0006 at 0.1 mg/kg, 1 mg/kg and 5 mg/kg in 41 children with cerebral malaria showed no difference in survival rate between the groups[15]. Also, 19 patients with refractory acute graft-versus-host disease (GVHD) were given infusions of another murine IgG1-κ mAb, B-C7, in a Phase I/II trial[16]. Of these patients, 74% had either a very good partial response or a partial response, although regression occurred after treatment was stopped and all but one patient died.

cA2 and other genetically engineered anti-TNF-α antibodies

More recently a number of chimeric anti-TNF-α antibodies have been developed using genetic engineering, and clinical trials have begun to assess their tolerability and efficacy in the neutralization of TNF-α. One of these, cA2 (developed by Centocor), was 'humanized' by replacing the constant region of a murine mAb, which binds to human TNF-α with high affinity, with its human counterpart[17]. The TNF-α binding and neutralization characteristics are therefore maintained, although immunogenicity still presents some problems because approximately a quarter of the chimera is still derived from the murine antibody[18].

Evidence that specific TNF-α blockade can be effective in inflammatory disease was shown in a randomized, placebo-controlled, double-blind study in which 73 patients with rheumatoid arthritis received either a single dose of cA2 (1 mg/kg or 10 mg/kg) or placebo administered by intravenous infusion over a 2 h period[19]. The antibody was well tolerated, and at the primary endpoint of 4 weeks striking improvements in disease activity criteria were observed: 19 of 24 (high-dose cA2), 11 of 25 (low-dose cA2) and 2 of 24 (placebo) patients achieved a Paulus 20% response, which is an amalgam of clinical (tender/swollen joints, pain/fatigue score, morning stiffness and grip strength), observational (disease severity: patient/observer) and laboratory variables [erythrocyte sedimentation rate (ESR), C-reactive protein (CRP)]. Over half of the patients on the high-dose regimen also passed the more stringent 50% Paulus criteria. Two severe adverse effects were noted: one patient receiving 1 mg/kg cA2 developed pneumonia that was deemed 'possibly' related to treatment, and one patient receiving 10 mg/kg had a fracture deemed 'probably not' treatment-related. The effects of repeated cA2 treatment administered during episodes of disease remission were also assessed in patients who had completed between two and four treatment cycles[20]. They showed maintained disease responses,

although there was a trend towards shortened response times. The treatment was safe and generally well tolerated, although half of the original patients developed low-titre human anti-cA2 responses, perhaps an indication of the need for concurrent immunosuppressive therapy. Regular cA2 treatment may achieve long-term disease suppression in patients with active rheumatoid arthritis, although limited efficacy of long-term treatment could be associated with accelerated clearance of infused cA2 or development of antibody responses that block binding of cA2 to TNF-α. There is evidence that cA2 has a major effect on the recruitment of macrophages and lymphocytes to the synovium by downregulation of cytokine-inducible vascular adhesion molecules[21–23]. Therefore, long-term anti-TNF-α treatment in these patients may interrupt the destructive process itself, as well as diminishing the inflammatory processes involved in its painful disease manifestation[18].

The cA2 antibody has also shown benefit when used to treat patients with active Crohn's disease, which is known to be associated with elevated TNF-α (Refs 24,25). An open label study in ten patients who received a single infusion of cA2 showed that the antibody was well tolerated and beneficial in the majority of patients[25]. Another recent study, in which six patients with advanced HIV-1 infection were given two infusions of cA2 (10 mg/kg), showed that the antibody was well tolerated and led to transient reduction in serum levels of immunoreactive TNF-α, although no changes in HIV RNA levels or CD4 counts were observed[26]. Serum TNF-α levels were reduced from a mean pre-infusion level of 6.6 pg/ml to 1.1 pg/ml 24 h after the first infusion, returning to baseline within 7–14 days, thus demonstrating a pharmacodynamic relationship between cA2 (half-life in serum of approximately 10 days) and serum TNF-α levels.

Another engineered humanized anti-TNF-α antibody, CDP571 (developed by Celltech), was constructed by grafting the complementarity determining region of a murine anti-TNF-α antibody to human IgG4 constant regions. This antibody, when administered in a single dose of 10 mg/kg (i.v.) (but not at 1 or 0.1 mg/kg), caused significant improvements in a double-blind, placebo-controlled study in 24 patients with active rheumatoid arthritis[27]. The antibody was well tolerated and led to improvements in disease markers (ESR and CRP) and disease activity score (number of tender/swollen joints and patient assessment score) during the 8-week follow-up period. A separate study also showed that CDP571 was well tolerated, and a dose-dependent decrease in serum TNF-α levels was observed, concomitant with a reduction in IL-1β and IL-6, when administered to patients with acute septic shock[28]. However, no conclusions on survival rates in these critically ill patients could be reached. The immunogenicity of this antibody appeared to be negligible, and only low levels of primarily IgM anti-CDP571 antibodies were detected which had no effect on TNF-α neutralization or antibody clearance[29].

MAK 195F is a third anti-TNF-α mAb that has been used: prophylactic administration to 21 high-risk patients before undergoing bone marrow transplantation was assessed during a Phase I/II trial[30]. Onset of acute GVHD was delayed in comparison with onset in 22 historical controls, and use of the antibody was not associated with any side effects. The same antibody has also been used in an attempt to treat patients with septic shock, although no beneficial effect on survival was noted[31].

Soluble TNF receptors as TNF-α antagonists

An alternative approach to that of anti-TNF-α antibody infusion is the use of naturally occurring TNF-α antagonists, namely the TNF receptors, to specifically limit its bioavailability. Recombinant soluble receptors constructed as multimeric Ig fusion proteins have improved affinity and half-life *in vivo*[18]. A recent double-blind, placebo-controlled trial of 180 patients with active rheumatoid arthritis used a recombinant human soluble p75 TNF receptor dimer, TNFR:Fc, fused to the Fc portion of IgG1 (Ref. 32). The data from the trial are not yet published, although the fusion protein was apparently efficacious, well tolerated and non-immunogenic[18].

Another TNF-α antagonist, Hoffmann-La Roche's Ro452081, is a recombinant chimeric molecule constructed by fusing the extracellular domain of the p55 TNF receptor to the hinge region of the IgG1 heavy chain. Results in animal models of allergic inflammation, using guinea-pigs and Brown Norway rats, have shown that Ro452081 is able to inhibit antigen-induced responses in the airways of sensitized animals[33]. Ro452081 (1–3 mg/kg, i.p.) decreased infiltrating neutrophil and eosinophil accumulation in bronchoalveolar lavage fluid and stopped antigen-induced microvascular leakage. However, at present no clinical data concerning the use of this compound in humans are available.

Thalidomide and its analogues

Thalidomide (α-*N*-phthalimidoglutarimide) is an immunomodulatory and anti-inflammatory drug that was originally used as a sedative, although it is now widely associated with

its unfortunate teratogenic and neurotoxic properties. This drug is now being reassessed, because it has been shown to be clinically useful in a number of conditions through its ability to selectively inhibit TNF-α synthesis[34]. However, reliable birth control methods must be used by women taking thalidomide, and monitoring for neurological effects is required in all patients. Andrulis Pharmaceuticals and Celgene Corporation in the USA are both involved in developing thalidomide for clinical use. FDA authorization has been granted for the use of thalidomide in a number of conditions, including multiple sclerosis, Crohn's disease and HIV infection.

Thalidomide is the drug of choice in the treatment of erythema nodosum leprosum, an acute inflammatory complication often seen in patients with lepromatous leprosy[34], and has also been used to treat patients with rheumatoid arthritis[35,36], HIV-associated aphthous ulceration[37] and chronic tuberculosis[38]. An early trial in patients with chronic GVHD indicated that thalidomide is safe and effective[39]. Also, a number of double-blind, placebo-controlled trials have indicated that thalidomide may be effective in the treatment of chronic diarrhoea and wasting associated with HIV disease[38,40,41]. Its use in the treatment of HIV-infected patients is also merited because raised serum TNF-α levels in these patients may play a role in the upregulation of HIV expression via the NF-κB transcription factor[42]. Indeed, thalidomide can inhibit HIV-1 replication in monocyte-derived macrophages. The mechanism whereby thalidomide inhibits TNF-α production is unclear, although there is evidence to suggest that it exerts its inhibitory effect by selectively increasing the rate of TNF-α mRNA degradation[43].

Thalidomide has been used in asymptomatic HIV-positive patients as well as in patients with AIDS. There are varied reports of adverse effects, including peripheral neuropathy, severe rash and somnolence. However, benefits in reversing HIV-associated wasting have been apparent, although systemic effects on immune parameters have not been shown[38,41]. Thalidomide given orally at 300 mg/day to symptomatic HIV-positive patients with and without associated tuberculosis infection did not inhibit serum TNF-α levels in HIV-infected patients, although there were modest reductions in TNF-α levels in patients with both infections[38]. The drug proved effective in promoting weight gain, although there was no demonstrable effect on CD4 count or viral load. Another study in symptomatic HIV-positive patients given four daily oral 100 mg doses showed a reversal of the wasting process; as with the previous study, little effect on CD4 count or viral load was found and serum TNF-α levels were not measured[41]. Finally, our own studies have shown that thalidomide at 100 mg/day has no apparent effect on systemic immune parameters in asymptomatic HIV-infected patients (J.B. Marriott *et al.*, unpublished). There was also no effect of thalidomide on patient weight, probably because wasting was not so advanced in these patients. There was, however, notable improvement in the chronic diarrhoea of patients who were treated off-study.

Side effects resulting from the use of thalidomide led to the withdrawal of seven out of ten (70%) patients in our study, mainly because of somnolence. This contrasts to only 7 out of 39 (18%) and 2 out of 14 (14%) in the higher-dose studies in symptomatic patients. This perhaps indicates lower tolerance of adverse effects in asymptomatic patients compared with ill patients. However, it may also reflect the longer duration of our study, which may have implications when considering longer-term usage of this drug in patients. The results from an open label trial of thalidomide in 25 patients with rheumatoid arthritis also emphasized the limits imposed by the side effect profile of this drug[44]. Patients who were able to tolerate a daily dose of 350 mg showed clinical benefit, whereas those who could not tolerate more than 250 mg did not. Another study on patients with rheumatoid arthritis had to be stopped after only 12 patients had been enrolled when it became clear that there were unacceptable side effects, including somnolence, peripheral neuropathy and severe rash, in the thalidomide group[45].

Absorption and solubility problems

Difficulties in drug absorption may account for poor systemic availability: patients treated with thalidomide for GVHD seem to demonstrate a gut mucosal dysfunction similar to that seen in patients with HIV infection. Furthermore, the poor solubility of thalidomide provides a barrier to good systemic bioavailability. Similar doses to those used in our study have been shown to be effective on aphthous ulcers in the gut[37] and in the treatment of patients with microsporidium infection[40], indicating that efficacy in treating gut-localized pathogenesis is not indicative of systemic bioavailability. The serum concentrations required to achieve systemic efficacy using an oral dose may not be possible without considerable toxicity. The levels of thalidomide in the blood of HIV-infected patients receiving the drug have not been determined previously, perhaps indicating that pharmacokinetic studies are needed to assess the dose–absorption balance.

Given the problems in administering an effective, nontoxic oral dose of thalidomide, there is interest in the design of compounds that are based on the thalidomide structure, but which have greater anti-TNF-α activity, are less toxic and have greater stability – thalidomide itself has a half-life in human plasma of under 2 h because of hydrolysis of its glutarimide ring (Figure 2). A large number of analogues, such as CC1069, CC1104 and CC1115 (Figure 2), have been generated by Celgene by introducing structural modifications in different moieties of the parent molecule[46,47]. Compounds with up to 400-fold greater activity than thalidomide have been made. At least one of these compounds, with an IC_{50} of approximately 1 μM (compared to ≈200 μM for thalidomide), is also completely water-soluble at 37°C (J.B. Marriott *et al.*, unpublished). This may be important in terms of enabling a systemic effect to be achieved with a nontoxic oral dose. These compounds, some of which are highly active in protecting mice from LPS-induced lethality, will soon be assessed in Phase I clinical trials[46].

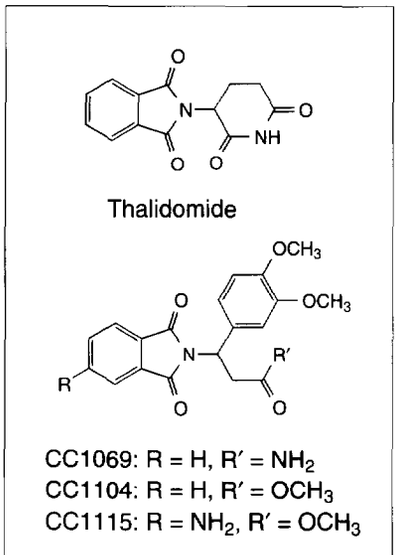


Figure 2. *Thalidomide and its analogues.*

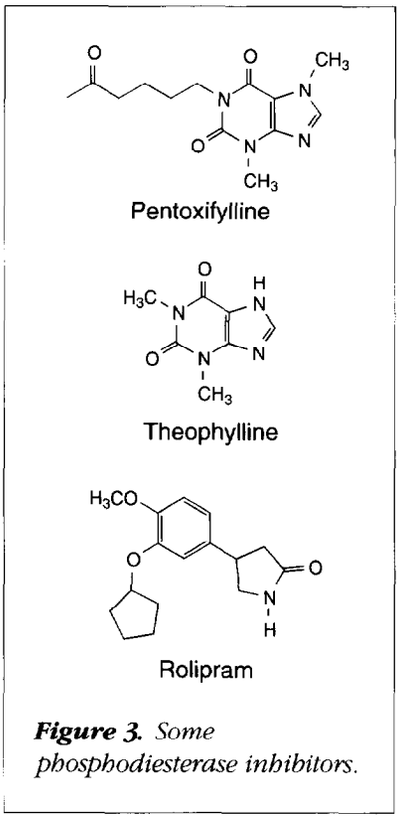


Figure 3. *Some phosphodiesterase inhibitors.*

Phosphodiesterase inhibitors

It is well established that the level of intracellular adenosine 3′,5′-cyclic monophosphate (cAMP) is an important factor in the inflammatory response to a variety of stimuli. Its role in the production of proinflammatory cytokines, such as TNF-α, and anti-inflammatory cytokines, such as IL-10, by macrophages is indicated by studies with agents that selectively affect its intracellular concentration. Agents that lead to increased cAMP levels, either by stimulating its formation or by preventing its breakdown, inhibit proinflammatory cytokines, such as TNF-α and IL-1β, and also Th1-type cytokines, such as gamma-interferon (IFN-γ) and IL-12, resulting in an immunosuppressive effect. Recently, compounds that are able to inhibit cAMP phosphodiesterase, the enzyme responsible for the breakdown of cAMP, have been suggested as anti-TNF-α agents. Phosphodiesterase type 4 (PDE 4) is thought to be the major isoform responsible for cAMP breakdown in inflammatory cells. Specific inhibitors of this isoform include rolipram [4-(3′-cyclopentyloxy-4′-methoxyphenyl)-2-pyrrolidone] and Ro201724 [4-(3-butoxy-4-methoxybenzyl)-imidazolidin-2-one] and its analogues developed by Glaxo Wellcome[48] (Figure 3). Interestingly, there is also evidence that some of the thalidomide analogues exert their anti-TNF-α activity via inhibition of PDE 4 (Celgene Corporation, pers. commun.). PDE 4 inhibitors are currently the most effective compounds for increasing cAMP levels and thereby inhibiting TNF-α (Ref. 49). Much of the work on these inhibitors, including assessment in clinical studies, has been done on non-specific phosphodiesterase inhibitors, such as the xanthine derivatives oxpentifylline (pentoxifylline) and theophylline (Figure 3). Early work showed that pentoxifylline treatment leads to a reduction of TNF-α mRNA accumulation by inhibiting transcription of the TNF-α gene[50].

Clinical studies of pentoxifylline

Initial observations indicated that pentoxifylline could inhibit serum TNF-α in healthy endotoxin-treated volunteers[51], and it also seemed to be of benefit to cancer patients[52], children with cerebral malaria[53] and recipients of bone marrow[54] and renal allografts[55]. This led to its application in at least three clinical trials, mainly in HIV-infected people, the results of which have recently been published. In the first trial, 25 patients with advanced AIDS were given 1,200 mg/day of pentoxifylline, and this led to decreased TNF-α mRNA in 10 of the 16 patients who finished the study[56]. However, the use of pentoxifylline in 31 AIDS patients treated for 8 weeks at a dose of 2,400 mg/day in an open label study led to half of the patients dropping out early[57]. Furthermore, no

improvements in activation markers, CD4 cell count or in wasting were seen in the treated group, nor was serum TNF-α decreased. Poor immune response to pentoxifylline treatment was also observed in a double-blind, placebo-controlled study in patients with early HIV disease and concurrent tuberculosis, who were given pentoxifylline at 1,800 mg/day for four months[58]. There was greater tolerance at this dose, but there was no effect on CD4 counts, wasting and activation markers, and only a non-significant trend towards TNF-α reduction. Interestingly, viral load was decreased in the treated patients compared with the placebo group, despite the absence of effects on the immune parameters studied.

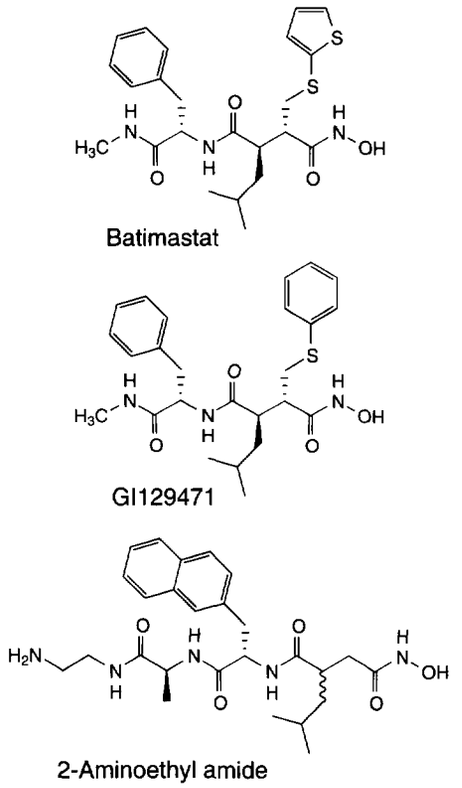


Figure 4. *Some matrix metalloproteinase inhibitors.*

These disappointing results may relate to dosage, lack of absorption, or may simply reflect the possibility that TNF-α modulation is apparent only at sites of inflammation. Another recently published report showed that 300 mg of intravenous pentoxifylline was not effective in treating patients with Jarisch–Herxheimer reactions, which are severe systemic inflammatory reactions associated with the antibiotic treatment of relapsing fever[59]. No inhibition of serum TNF-α or any of the associated clinical manifestations were observed, even though this method and dose of administration have been shown to inhibit TNF-α in response to endotoxin[51]. It is possible that variation in drug efficacy between these studies may be the result of differences in the cell populations and/or stimuli involved in the inflammatory response.

The evidence from clinical studies with pentoxifylline to date suggests that more potent TNF-α phosphodiesterase inhibitors are required to achieve notable systemic effects in humans at doses that are tolerated. Pentoxifylline analogues, presumably with increased potency and less toxicity, have been developed and are currently being assessed in animal models. However, the use of specific PDE 4 inhibitors may prove to be more selective and provide greater therapeutic potential[60]. For example, rolipram, which has 500-fold greater potency than pentoxifylline, was recently shown to be effective in experimental autoimmune encephalomyelitis, a rat model of multiple sclerosis[61]. No data are yet available from clinical trials, although in human *in vitro* studies efficacy has been demonstrated[62].

Other PDE 4 inhibitors

A number of other potent and selective PDE 4 inhibitors also show promise. BRL61063 [1,3-di(cyclopropylmethyl)-8-amino-xanthine], developed by SmithKline Beecham, is a PDE 4 inhibitor that inhibits serum TNF-α in LPS-treated D-galactosamine-sensitized mice (IC_{50} = 0.1 mg/kg), corresponding with protection from lethality[63]. RP73401 has been used to treat rheumatoid arthritis in a double-blind, placebo-controlled trial of 35 patients, who received either 200 μg or 400 μg/day for 28 days. A trend towards improvement in terms of number of tender joints and other assessments of disease activity was associated with reduction of CRP and IL-6, although TNF-α remained unchanged[64]. Also, CDP840, one of a series of triarylethanes developed by Celltech, has been put forward as a novel treatment for asthma. Its use on guinea-pigs demonstrated its ability to inhibit ozone-induced airway hyperresponsiveness and noncholinergic bronchoconstriction[65].

Matrix metalloproteinases

The matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes that are involved in the degradation of connective tissue. Subfamilies of MMPs can be loosely defined in terms of substrate specificity for particular extracellular components such as laminin, collagen and fibronectin. An extensive review of MMP inhibitors has recently been published[66]. The structures of some MMP inhibitors are shown in Figure 4. Of relevance to this review are two observations: (1) MMPs were shown to protect mice against a lethal dose of endotoxin[67]; (2) MMP inhibitors block processing of soluble TNF-α from its

membrane-bound precursor form, implying that MMPs are involved in TNF-α maturation[68,69]. The identity of this 'TNF convertase' remains to be determined. It is possible that more than one MMP contributes to this activity, especially since many of these enzymes are overexpressed in diseases such as arthritis and cancer, although there is also evidence suggesting that the main physiological activity of this 'convertase' may be attributable to non-MMP[70]. Under disease conditions, it is likely that MMP activity is increased as a result of a breakdown in homeostasis between enzyme activity and that of locally produced tissue inhibitors of metalloproteinases (TIMPs), leading to tissue degradation[71]. Therefore, compounds that either downregulate MMPs or upregulate TIMPs may prove effective in inhibiting the processing of TNF-α.

In addition to their effect on membrane-bound TNF-α, MMPs also appear to be responsible for the processing of TNFR (75 kDa). Indeed, one MMP, TAPI, blocks the shedding of both TNF-α and TNFR (75 kDa)[72], perhaps indicating that these molecules may be coordinately regulated. Therefore, any decrease in soluble TNF-α production may be mirrored by the increased sensitivity of cells to soluble TNF-α and to unprocessed membrane-bound TNF-α, which is also bioactive. Although clinical trials of current MMP inhibitors are under way – Batimastat (BB94; Figure 4) is being assessed in cancer patients – it is important to identify other inhibitors that selectively block TNF-α processing for future clinical assessment in TNF-α-mediated disease.

Adenosine agonists

Adenosine is an endogenous purine nucleoside produced as a result of intracellular degradation of the metabolite ATP (Figure 5). In conditions of metabolic stress, massive catabolic depletion of ATP leads to the localized release of micromolar concentrations of adenosine, which in turn exerts a downmodulatory effect on a number of leukocyte-mediated inflammatory processes via its binding to one of four known receptors (A_1, A_{2A}, A_{2B}, A_3). These effector mechanisms are usually beneficial, although, in some circumstances, such as septic shock, excessive cytokine production, leukocyte infiltration and oxygen radical formation may cause tissue damage. An early report established adenosine-based compounds as possible TNF-α antagonists in inflammatory disease, although there is some uncertainty as to whether A_2 or A_3 receptors are the more important targets in this respect[73,74]. One adenosine analogue, 2-chloroadenosine, which reduces TNF-α mRNA levels,

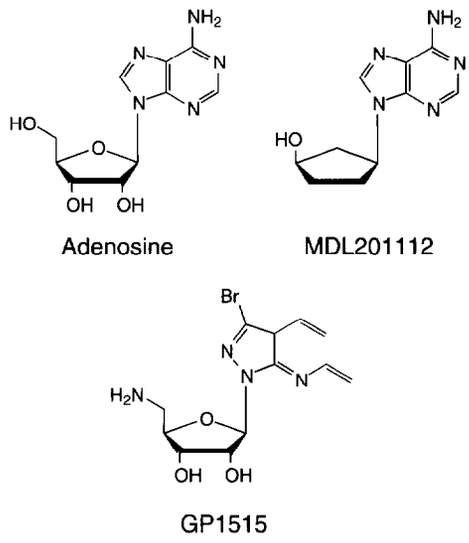
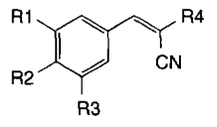


Figure 5. *Chemical structure of adenosine and adenosine analogues.*

binds to all receptor subtypes[74]. Adenosine and another related carbocyclic nucleoside analogue, MDL201112 (Figure 5), were shown to inhibit TNF-α production by activated mouse peritoneal macrophages[75]. Furthermore, a single dose of MDL201112 protected over 90% of mice given a lethal dose of LPS and inhibited the appearance of serum TNF-α. However, adenosine itself was not effective, probably because of its rapid metabolism *in vivo*. Adenosine analogues display impressive specificity characteristics, inhibiting TNF-α expression but not affecting the other macrophage-specific cytokines, IL-1β, IL-6 and IL-8 (Ref. 74).

The therapeutic use of exogenous adenosine and its analogues has unfortunately been limited to date by severe cardiovascular side effects[76]. However, in order to take advantage of the benefits of adenosine and reduce the possibility of systemic toxicity, an alternative approach has focused on the use of agents that are able to increase endogenous production of adenosine. Specific inhibitors of adenosine kinase have been developed that prevent phosphorylation of adenosine to AMP, thereby increasing the amount of adenosine that can be transported out of the cell to act locally[77]. The short half-life of adenosine should ensure that only cells in the local environment are affected and, therefore, systemic toxicity is kept minimal. One such inhibitor, GP1515 [4-amino-1-(5-amino-5-deoxy-1-β-D-ribofuranosyl)-3-bromopyrazolo(3,4-D)pyrimidine; Figure 5], significantly

decreased mortality in mice given a lethal dose of endotoxin, an effect that could be reversed by an adenosine receptor antagonist, thereby confirming endogenous adenosine as the protective factor[77]. Plasma levels of TNF-α, but not of IL-1β or IL-6, were also lower in treated animals, again underlining the specificity of the inhibitor – a factor that may prove to be important for clinical use.



General structure

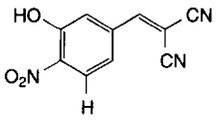


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Figure 6. *Chemical structure of tyrphostins.*

Protein kinase inhibitors

The phosphorylation of tyrosine residues by protein tyrosine kinases (PTKs) is a crucial process that enables signal transduction in response to a massive array of physiological stimuli. The cellular processes of proliferation, differentiation and signalling are regulated by receptor tyrosine kinases, which participate in transmembrane signalling, and intracellular tyrosine kinases, which transduce these signals to the various effector mechanisms. Enhanced activity of PTKs has been implicated in a number of diseases, including cancer, and in diseases associated with the inflammatory response, such as septic shock[78]. A number of reports have demonstrated that LPS stimulation of macrophages leads to protein tyrosine phosphorylation and the subsequent release of inflammatory mediators, including TNF-α – a response that can be inhibited by PTK inhibitors[79–81]. TNF-α-induced tyrosine phosphorylation in target cells, which is thought to result from the activation of Src-type PTKs such as Hck and Lyn[82], can also be blocked by PTKs. It is thus possible that TNF-α-mediated action can be blocked not just by preventing its action but also by inhibiting its production when induced by LPS.

The transcriptional activation of a number of target genes by extracellular signalling proteins, such as cytokines, is achieved via the JAK-STAT (signal transducer and activation of transcription) pathway. The specific activation of different family members of the JAK tyrosine kinases and cytoplasmic STAT transcription factors enables the generation of a diversity of cellular responses to various ligands. The identification and targeting of specific JAKs involved in TNF-α signalling may provide a novel mechanism for inhibition of inflammatory responses, and evidence that cytokine activity can be modulated by interfering with these signalling pathways has recently emerged[83]. Because PTKs are involved in each step of the intracellular signal cascade, the characterization and selective inhibition of specific enzymes involved in TNF-α-specific pathways and in pathways leading to TNF-α production may be possible.

A number of naturally occurring PTK inhibitors, such as herbimycin A and genistein derived from fungal extracts, have served as the starting point for the development of synthetic inhibitors. These include a group of inhibitors called tyrphostins, which are similar in structure to tyrosine, hence their name (Figure 6). One of these compounds, AG126, has been shown to prevent LPS-induced death in mice, a feature that correlated with reduced TNF-α and nitric oxide (NO) production[84], although more recent data indicated that AG126, as well as two other synthetic tyrphostins, were unable to inhibit TNF-α or NO in the RAW 264.7 murine macrophage line[85]. TNF-α alone is not responsible for the lethal effects of LPS in mice, since other effectors such as NO, IL-1β and IFN-γ are also involved. In this respect, it is interesting to note that two other tyrphostins, AG490 and AG556, are more active inhibitors of specific TNF-α effects[84]. There are many other groups of PTK inhibitors, including the pyridinylimidazoles, which have been shown to inhibit TNF-α and IL-1 production in human monocytes[86]. However, the design of PTK inhibitors for TNF-α-mediated pathways alone could be an important finding because it suggests that inhibitors of PTK activity that target particular steps along TNF-α signalling pathways may become available.

TNF-receptor-associated factors

Another possible site for anti-TNF-α intervention is the recently discovered family of signal transducers, the TNF-receptor-associated factors (TRAFs)[87]. These proteins share a conserved C-terminal TRAF domain and are associated with the cytoplasmic domain of the 75 kDa TNF receptor and CD30. Differences in the TRAF-binding patterns of these TNF receptor family proteins have been shown[88], indicating a possible mechanism for signalling specificity. As more becomes known about TNFR-mediated signalling through these proteins, the greater the possibility that intervention strategies can be developed.

Many groups of compounds have shown the potential to inhibit TNF-α overproduction. New compounds derived from existing structures have been designed with the aim of increasing specificity and reducing toxicity, and are showing

promising results in a number of animal models of disease in which elevated TNF-α is associated with pathogenesis. Furthermore, novel compounds are being developed as a result of our increased understanding of the mechanisms involved in TNF-α production and activity. As this process continues, we can expect drugs of greater specificity to emerge, especially compounds that target individual kinases along the intracellular signal transduction pathway. As these new classes of compounds move into the clinic, we can anticipate marked reductions of disease activity in a wide variety of conditions, underlining the importance of maintaining the fine balance between essential TNF-α-mediated host defence and pathogenesis.

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