Therapeutic modulation of cytokines

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The potent ‘proarthritic’ effects of cytokines such as interleukin-1 (IL-1), IL-6, tumour necrosis factor α (TNF α) on cells and isolated connective tissues,¹ the ability to induce synovitis by local injection of cytokines,² and the ability to block the pathology of experimental models of arthritis by anticytokine reagents^{3–4} strongly argue that these potent local hormones play a key role in the pathology of chronic arthritic conditions such as rheumatoid arthritis (RA) and septic arthritis. It is not surprising, therefore, that the cytokines are perceived as major therapeutic targets in these conditions. The first faltering attempts to develop anticytokine agents started only a short time ago, in the mid 1980s, but through the growth in the biopharmaceutical industry in the past decade we currently have a number of anticytokine agents in clinical trials. This paper will consider briefly the methods used to modulate cytokines, and interested readers should refer to recent reviews for more details.^{5–6}

Therapeutic modulation of cytokines

Four major approaches to therapy can be taken: inhibit cytokine synthesis; inhibit cytokine release; inhibit cytokine action; inhibit cytokine intracellular signalling pathways. Table 1 shows the various points of intervention, which will be considered in turn.

INHIBITION OF CYTOKINE SYNTHESIS

This can be achieved at a number of levels. The first is blockade of the intracellular signalling pathways which result in the transcription of individual cytokine genes. In the past few years it has been demonstrated that the potent immunosuppressants cyclosporine and FK506 inhibit the synthesis of lymphokines such as IL-2, IL-3, IL-4, and interferon gamma in this manner by forming a complex with specific cellular binding proteins which then inhibits the calmodulin-dependent protein phosphatase,

calcineurin. The function of calcineurin is believed to be to dephosphorylate a subunit of the lymphokine transcription factor, nuclear factor of activated T cells, which can then enter the nucleus and form the active transcriptional complex to switch on the genes transcribing IL-2 and related cytokines. The formation of the drug-binding protein complex inhibits this dephosphorylation and thus the cell fails to generate the transcriptional complex.⁷ Given its potent immunosuppressive activity, cyclosporine is surprisingly ineffectual in treating RA. This strengthens the argument that lymphokines play a minor role in the pathology of established RA.⁸ Our understanding of the mechanism of action of these immunosuppressants should allow the development of selective agents for blocking the postreceptor/pretranscriptional intracellular signalling pathways involved in cytokine synthesis.

Oligonucleotide based drugs—There is enormous interest in agents which can selectively inhibit gene transcription or mRNA translation. Such agents are generally modified oligonucleotides with nuclease resistant bonds. Antisense oligonucleotides designed to hybridise with the AUG translation initiation codon have been the most popular choice, and the synthesis of a wide range of cytokines and cytokine receptors in vitro has been reported,⁹ one group has shown that antisense probes to the murine type I IL-1 receptor have anti-inflammatory activity in vivo.¹⁰ Shiozawa and Hino (this symposium) report that down-regulation of the transcription factor AP-1 by oligonucleotides inhibited synovial expression of IL-1 and IL-6 and the symptoms of collagen-induced arthritis in mice.¹¹ This is a very active area of pharmaceutical research and development, with a number of oligonucleotide based drugs in early phase clinical trial.⁹

Low molecular mass inhibitors—There are a growing number of low molecular mass compounds reported to inhibit the synthesis of cytokines (table 2). Most are active in the micromolar range and are therefore potential therapeutic agents. These compounds generally have an unknown mechanism of action. Tenidap is one of these compounds which has been claimed to inhibit IL-1 synthesis. This agent is now in clinical trial in RA and has been reported to show clinical effects consistent with the inhibition of cytokine synthesis.¹² The only tenuous common association shown by most of these molecules is the ability to inhibit arachidonic acid oxidation. It is not clear, however, if this property is related to inhibition of cytokine synthesis.

Table 1 How do you inhibit cytokines therapeutically?

1.	Inhibit cellular activation pathways leading to cytokine synthesis
2.	Inhibit the transcription of cytokine genes
3.	Inhibit the splicing/metabolism of cytokine gene mRNA
4.	Use oligonucleotide based agents to inhibit cytokine mRNA translation
5.	Inhibit the proteolytic processing of cytokines
6.	Inhibit selective cytokine release pathways
7.	Block the action of cytokines by use of neutralising monoclonal antibodies or soluble cytokine receptors
8.	Antagonise the binding of cytokines to their cognate receptors
9.	Inhibit cytokine specific intracellular signalling pathways
10.	Use antagonist cytokines to downregulate cytokine induced cellular activation

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Table 2 Low molecular mass inhibitors of cytokine synthesis

Compound	Cytokine inhibited
Glucocorticoids	Many cytokines
Prostanoids	IL-1, TNF
Taurolidine	IL-1
SK&F 105809	IL-1
SK&F 86002	TNF
Tenidap (Pfizer)	IL-1
Pentamidine	IL-1
IX (Sandoz)	IL-1
Antioxidants	IL-1, IL-6, TNF
Substituted chalcones	IL-1, TNF
E5090 (Tsukuba)	IL-1
E3330 (Tsukuba)	TNF
SA 3443 (Santen)	TNF
RP54754	IL-1
RPR102194	IL-1
TA 383	IL-6
Immunosuppressants	Lymphokines
Sodium aurothiomalate	IL-8
Antiarthritic compounds	Cytokines/lymphokines
Vitamin D-3	IL-8
Vitamin K	IL-6

INHIBITION OF CYTOKINE RELEASE

If it does not prove possible to block the transcription or translation of a cytokine, it may prove possible to block its release from the producer cell.

Inhibitors of cytokine processing—Certain cytokines such as the key proinflammatory molecules IL-1 β and TNF α require proteolytic processing to produce the active form. This processing is achieved by the action of specific proteases. The enzyme responsible for the conversion of the 33 kDa IL-1 β precursor to the active 17 kDa form is termed IL-1 β converting enzyme (ICE), and a number of companies including MSD, Sterling Winthrop and Vertex have developed potent inhibitors and reported activity in vivo.¹³ The recent determination of the structure of ICE at 0.26 nm resolution should help in the design of inhibitors.¹⁴ Interestingly, it has been shown that gold thiomalate and auranofin are non-competitive inhibitors of ICE.¹⁴ Several reports have recently appeared demonstrating that the TNF α precursor is cleaved by a zinc dependent metalloendopeptidase and can be cleaved by members of the matrixin family. Inhibitors of these enzymes can inhibit TNF release and such inhibitors have activity in vivo.^{15–17} Thus it appears that it may be possible both to inhibit the release of TNF α and inhibit the activity of tissue degrading metalloproteases using the same inhibitors.

Inhibition of cytokine release—In addition to requiring processing, IL-1 and a few other

cytokines lack signal sequences required for export and are released from cells by a novel pathway which could present a selective therapeutic target.¹⁸ A few compounds have been reported to inhibit IL-1 release, including pentamidine isethiocyanate (an antiprotozoal agent) and IX 207,887 a compound developed by Sandoz.⁶ In animal models, IX 207,887 demonstrates anti-inflammatory, antipyretic, antinociceptive and antiarthritic activities.¹⁹ In a double-blind clinical trial in patients with RA, it demonstrated significant anti-inflammatory and antiarthritic activity, although the trial was too short to determine if there was any inhibition of the progression of joint damage.²⁰ Sandoz have also reported the synthesis of tri- and tetrapeptides which inhibit the release of IL-1 β with in vitro IC₅₀ values of 0.1 μ mol/l.

INHIBITION OF CYTOKINE ACTION

The inhibition of cytokine synthesis and release is still proving difficult to achieve, and few compounds have reached the stage of clinical trial. In contrast, there are a number of agents in clinical trial which are aimed at cytokines which have been produced and released into the extracellular fluid. Two major forms of therapeutic modality are under investigation: agents such as neutralising monoclonal antibodies and soluble cytokine receptors which bind to the cytokine in the fluid phase and sterically inhibit its binding to the receptor, and antagonists of cytokine binding to its cell surface receptor. In addition, a third modality—'antagonist' cytokines—is under experimental investigation.

Cytokine-neutralising monoclonal antibodies—Monoclonal antibodies (MAbs) have a number of advantages as therapeutic agents: they can be raised to any cytokine target; their natural milieu is the blood, extracellular fluids, and mucosal surfaces; they have long circulating half lives; and their physical properties of size and affinity can be modified to the therapeutic demand. However, they have one major disadvantage—they are raised in rodents, and produce a strong immune response in human recipients. This has now been overcome by the generation of chimaeric antibodies (mouse variable region and human Fc) and humanised antibodies, in which only the antigen binding hypervariable regions are murine.²¹ There is now a significant body of experimental evidence to support the idea that antibody based neutralisation of cytokines can inhibit tissue pathology (table 3). Anti-TNF α MAbs have been assessed in patients with septic shock with, so far, rather disappointing results.²² One open trial of a chimaerised anti-TNF α MAb, produced by Centocor, in the treatment of RA has produced a great deal of excitement, with clinical benefits being reported;²³ a placebo controlled double blind trial has confirmed the initial results. Other centres in London are testing the Celltech anti-TNF α MAb in double blind dose ranging studies. A note of caution should be introduced at this point. The neutralisation of TNF α in rodent models of

Table 3 Actions of cytokine neutralising antibodies

Cytokine neutralised	Disease model
IL-1	Antigen induced arthritis, endotoxin shock
TNF α	Many and varied including endotoxin shock, EAA, contact hypersensitivity, arthritis, Schwartzmann reaction
IL-3	Helminth induced mastocytosis
IFN γ	Schwartzmann reaction, endotoxin shock, arthritis, cerebral malaria
IL-3 and GM-CSF	Cerebral malaria
IL-4	Leishmaniasis
IL-5	Helminth induced eosinophilia
IL-6	Endotoxin shock
PDGF	Atherosclerosis
TGF β	Fibrosis

EAA = Experimental allergic encephalomyelitis; GM-CSF = granulocyte macrophage colony stimulating factor; IFN = interferon; IL = interleukin; PDGF = platelet derived growth factor; TGF = transforming growth factor; TNF = tumour necrosis factor.

arthritis such as adjuvant and collagen induced arthritis inhibits synovitis and tissue damage;²⁴ in contrast, the neutralisation of this cytokine fails to inhibit tissue damage in antigen induced arthritis in mice and rabbits.²⁵ These discrepant results may reflect the different response of these experimental lesions to non-steroidal anti-inflammatory drugs (NSAIDs). Adjuvant and collagen arthritis are largely cured by NSAIDs, while this class of drug has no effect on the progressive damage to articular cartilage and bone damage in antigen induced arthritis models—a reflection of the clinical experience with these drugs. As TNF α is a potent inducer of cyclo-oxygenase II and thus stimulates prostanoid synthesis, the neutralisation of this cytokine would be likely to inhibit prostanoid formation, and this could be responsible for some of the reported effects. We have found that a neutralising MAb to rabbit TNF α has a profound anti-inflammatory effect in animals with antigen induced arthritis, complete inhibition of joint swelling occurring within one week of treatment—an effect we have seen with no other drug tested. However, these animals showed no inhibition of cartilage proteoglycan loss.²⁵ This substantiates the growing belief that tissue damage and inflammation in the rheumatoid joint may not be causally linked.²⁶

Two additional problems are faced by antibodies. First, if articular cartilage chondrocytes are producing the cytokines which, acting in an autocrine fashion, stimulate the degradation of cartilage, then neutralising antibodies may not be able to penetrate the cartilage to switch off synthesis. This may be one explanation for our failure to inhibit cartilage damage in the rabbit. Second, with fully humanised antibodies the prolonged administration of large amounts of a particular idiotope is likely to induce an anti-idiotypic response. Of particular concern is the possibility that some of these anti-idiotypic antibodies could mimic TNF α and thus exacerbate the disease.

One exciting development which could overcome these various problems is the work of Saragovi's group, who have reported the synthesis of low molecular weight non-peptide mimetics of antibody combining sites.²⁷ If this proves to be a universally applicable strategy, antibodies would simply become the first step in the development of low molecular weight antagonists.

Soluble cytokine receptors—A second approach to the inhibition of cytokines in the extracellular milieu is to use soluble forms of cytokine receptors. This is one strategy developed by 'Mother Nature' to control cytokines. Such receptors have a higher binding affinity than antibodies, but suffer from having a short circulating half life. However, by linking an antibody Fc region to soluble receptors, the circulating half life can be considerably extended;²⁸ a number of investigators are producing such hybrid molecules with multiple binding sites which should enhance cytokine binding. Soluble receptors have been shown to suppress a range of experimental models of tissue pathology.⁶ Soluble IL-1 receptor was

shown to inhibit local allergen induced inflammation in human volunteers with no side effects being reported.²⁹ Hoffman La Roche are currently in early phase clinical trials in RA with a soluble TNF receptor-IgG Fc complex which is apparently showing clinical efficacy (Fenner, personal communication).

Cytokine antagonists—When pharmacologists think about inhibiting the action of an agonist, their first thought is to develop a receptor antagonist. Such ideas initiated the drive to develop anticytokine therapeutic agents, but in spite of extensive and expensive research no pharmaceutical company has yet devised a cytokine receptor antagonist. The reasons for this probably relate to the enormously high affinities of binding between cytokines and their receptors and the multi-point attachment of these two cognate proteins. However, Mother Nature has had many aeons of pharmaceutical experimentation and has developed an antagonist of IL-1. This protein, interleukin-1 receptor antagonist (IL-1ra), shares only 20–30% homology with the IL-1s, but is now regarded as the third member of the IL-1 family and binds to the functional type I IL-1 receptor without stimulating an agonist response. It is therefore a useful probe of the residues in IL-1ra which trigger receptor mediated cellular activation.³⁰ First described in the late 1980s, and cloned and expressed in 1990,³¹ during the past four years IL-1ra has been shown to be a potent inhibitor of many experimental models of human disease and, in particular, septic shock⁶ and has been tested in clinical trials for various conditions. It has been shown to ameliorate rodent models of arthritis,³² but had no effect on the induction³³ or (Lewthwaite, in preparation) chronic phase of antigen induced arthritis in the rabbit. However, it has proved to be a potent antifibrotic agent in both lung³⁴ and joints (Lewthwaite, in preparation) in animal models of lung fibrosis and arthritis, respectively. IL-1ra is in clinical trial for use in RA, but is unlikely to be of therapeutic benefit as it has a very short half life in the circulation. Indeed, at the time of writing Synergen, who have pioneered IL-1ra under the trade name Antril, have suffered a considerable setback with the failure of their second phase III trial of Antril for septic shock.³⁵ It is the author's view that a novel approach to agonist receptor interactions is required in order to develop clinically useful cytokine antagonists.

Antagonist cytokines—Cytokines are paracrine and autocrine mediators and, as such, form complex interacting networks capable of either stimulating or inhibiting cellular activity. The capacity for both actions probably depends on the initial state of the system. It has emerged in the past few years that certain cytokines act to downregulate the cellular activation induced by other cytokines. Molecules such as IL-4, IL-10 and transforming growth factor β have been shown to inhibit experimental conditions including inflammatory lesions and experimental models of arthritis.^{6, 36} The problem inherent in the clinical use of this therapeutic approach is that the administration of these

cytokines to healthy animals can, in itself, induce pathology. Much more information is needed to determine if it is possible to use such antagonist cytokines therapeutically in diseases such as RA.

Inhibition of cytokine induced intracellular signalling—The effects of cytokines are the result of specific upregulation of receptor mediated intracellular signalling pathways. Blocking these pathways inhibits the action of the cytokine. The mechanism of action of the immunosuppressants cyclosporine and FK506 has already been discussed. Many pharmaceutical companies have therapeutic programmes to develop inhibitors of cytokine induced intracellular signalling. Perhaps the most advanced is the programme to develop inhibitors of tyrosine kinases, which are increasingly seen to be involved in cytokine generated cellular activation. A number of bacterial metabolites such as genistein and erbstatin are known inhibitors of tyrosine kinases. Erbstatin has been used to develop a family of rationally designed tyrosine kinase inhibitors termed tyrphostins,³⁷ which have been shown to inhibit experimental septic shock³⁸ and are currently in clinical trial for psoriasis.³⁷ Parke-Davis scientists have recently reported the synthesis of a specific inhibitor of the epidermal growth factor receptor tyrosine kinase. The K_i value of this compound is an amazing 5 pmol/l.³⁹ Other compounds such as lisofylline, an inhibitor of phosphatidic acid which blocks cell activation by pro-inflammatory cytokines, are also in early phase clinical trials.

Conclusions

The past decade has seen enormous strides in our understanding of the biology of cytokines and their role in human disease. We are already seeing the introduction into the clinic of therapeutic cytokines such as interferons gamma and alpha, erythropoietin, and colony stimulating factors. The next decade should see the clinical use of cytokine modulators which downregulate the action of selected cytokines. The greatest efforts in this respect have been devoted to the treatment of septic shock—an acute condition believed to be caused by endotoxin driven production of proinflammatory cytokines such as IL-1 and TNF α . The acute nature of this disease and the experimental finding that septic shock in rodents, lagomorphs and primates could be blocked by single antiendotoxin or anti-cytokine agents gave the biopharmaceutical industry confidence that it could produce effective therapeutic agents; the experience so far is cautionary. As described by Wright,³⁵ six biopharmaceutical companies have now failed in their efforts to develop clinically effective therapies for this condition. They include well known companies such as Centocor, Synergen, Chiron, Xoma, and Immunex. In view of the great complexity of the pathology of rheumatoid arthritis, our expectations that blocking single cytokines (by complex agents such as cloned chimaerised antibodies or

soluble receptor-antibody complexes) can inhibit tissue pathology should remain at a sensible low level. Only time will tell if it is possible to inhibit complex cytokine induced pathology in man by removing single cytokines.

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