Prospects for the development of small molecular weight compounds to replace anti-tumour necrosis factor biological agents

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Tumour necrosis factor (TNF) blockade, achieved by the soluble receptors and antibodies, has been a major success in the treatment of rheumatoid arthritis (RA). The success of this treatment has also established biological agents as a new weapon in the armoury of therapeutic approaches to the treatment of autoimmune diseases. Established data and emerging evidence suggest that anti-TNF biological agents will have a therapeutic role in other autoimmune diseases. Infliximab is already licensed for Crohn’s disease and, recently, anti-TNF biological agents have shown major promise in spondyloarthopathies, psoriatic arthritis, and juvenile idiopathic arthritis. There is growing evidence that TNF blockade may be important in many other diseases, including sarcoidosis and psoriasis. Therefore, although the number of patients treated with the various anti-TNF biological agents is rapidly approaching half a million, there is no reason to suggest that the patient pool is anywhere near saturation. Rather, one can expect the number of patients with diseases likely to benefit from this form of treatment to increase.

However, there are problems with anti-TNF biological agents that limit their use. From a safety perspective, although TNF blockade has not demonstrated the massive problems of susceptibility to infections once feared, there are clearly problems, especially with patients who have latent tuberculosis. There is also the constant problem associated with using proteins as drugs of their administration and dosing. However, by far the biggest constraint in using anti-TNF biological agents is the cost of treatment, which is of the order of $15 000/patient/year. This has led to a relatively low uptake of the treatment in several European countries and to patients being described as “economic failures” in America. One must also add that the difficulty in producing proteins on a mass scale has led to a supply problem, especially for Enbrel.

THE CASE FOR SMALL MOLECULAR WEIGHT INHIBITORS

Despite the success of anti-TNF biological agents the major drawbacks of the treatment, especially the cost, have led to a major drive for a hopefully “cheaper” alternative. The main hope has been to find ways of blocking TNF production or functions with small molecular weight inhibitors that are orally bioavailable and, importantly, cheap to make. If such a drug were to be developed it would easily undercut the biological market with its lower cost and ease of delivery, assuming that it did not have an adverse safety profile. However, since the identification of TNF as a key therapeutic target for RA in the early 1990s there has been little evidence of a successful small molecular weight TNF blocker reaching the market. Why is this the case? Several inviting targets for a small molecular weight inhibitor that either blocks TNF function or production seem to exist, but there are major problems (Box 1): the amenability of targets for modulation by a small molecular weight drug (druggability), the certainty that a potential target is important in the disease process, the general problems of specificity and selectivity of both targets and drugs, and their respective relationship to mechanism based and non-mechanism based toxicity. Additionally, the need to find a drug with the right pharmacokinetic characteristics has made this particular Holy Grail difficult to obtain.

AMENABILITY OF A TARGET FOR A SMALL MOLECULAR WEIGHT DRUG

The sequencing of the human genome has indicated the existence of about 30 000 genes. The question that this poses to the pharmaceutical industry is how many of these genes are related to disease processes and, in addition, how might be functionally modulated by small molecular weight inhibitors. Potentially, the use of antisense oligonucleotides or the

Box 1: Parameters for the development of small molecular weight inhibitors of TNF production/function

- Is the target involved in the disease pathology? Has the target been validated in an appropriate disease related system? It may be desirable to validate the target in more than one such system if a wider range of disease targets is being contemplated. Also, is the function of the target potentially redundant such that other related proteins might take over its activity?
- Will the target be amenable to modulation by a small molecule? This generally requires the target to be an enzyme of some kind.
- How many biological processes comprise the target and which of those are unrelated to the disease process? Does the target have multiple biological roles such that inhibition would lead to unacceptable levels of mechanism related toxicity?
- Can an inhibitor/modulator be made selective enough? Given the structural homology between similar classes of enzymes (for example, kinases), the possibility of the inhibitor affecting molecules other than the intended target must be considered. Possibly, also, a small molecular weight compound might have effects on multiple classes of proteins that are structurally unrelated.

Abbreviations: BAL, bronchoalveolar lavage; BTK, Bruton’s tyrosine kinase; ELISA, enzyme linked immunosorbent assay; IKK2, IκB kinase 2; LPS, lipopolysaccharide; MAP, mitogen activated protein; moi, multiplicity of infection; NIK, NF-κB-inducing kinase; RA, rheumatoid arthritis; TNF, tumour necrosis factor

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more recently developed RNAi technology has the ability to reduce the expression of any gene regardless of structure or function in a highly selective way, but these approaches do not at present overcome the problems of delivery when applied to disseminated diseases such as RA. So far only one antisense drug has been approved for ocular administration in Cytomegalovirus retinitis, although, possibly, future developments may overcome these problems and make nucleic acid based drugs a better therapeutic proposition.

If on the other hand, the discussions are limited to small molecular weight chemicals then the structure and function of the target becomes a key consideration. The easiest and simplest approach to modulating TNF would be the design of small molecular weight inhibitors that would block TNF binding to its receptor (TNFR) in a manner not dissimilar to an anti-TNF biological agent. However, the quest to find a small molecular weight inhibitor with sufficient affinity to block TNF/TNFR interactions has not been successful. The major factor is probably the large footprint of interactions between the cytokine and receptor that would make it very unlikely that a small molecule could disrupt enough of the TNF/TNFR interactions to produce an inhibitor with sufficiently high affinity to be therapeutically useful. It is, however, worth noting that the blockade of receptor interactions with small molecules is feasible as is the case with the serpentine super family of receptors, which often also bind small molecules as well as proteins (for example, chemokines). The problem of blocking protein/protein interactions has therefore led the major effort towards generating small molecular weight inhibitors to fall on more conventional targets—that is, enzymes such as kinases, phosphatases, or proteases, etc.

**IDENTIFICATION OF “DRUGGABLE” TARGETS INVOLVED IN TNF BIOLOGY IN RA**

The major source of enzymes that might be amenable to modulation by small molecular weight compounds is potentially found in the signal transduction pathways involved in TNF production and function. In recognition of this there has been a major effect by both academia and industry over the past decade or so, to identify these pathways. Many of the molecules involved in transducing the signal from the TNF receptor to NF-κB have been elucidated. Similar work has been pursued in studying the signalling mechanisms controlling TNF production. In this case, because the primary stimulus of inducing cytokine production in RA is unknown, responses to lipopolysaccharide (LPS) have been often used as a surrogate. However, despite the many studies performed, only two targets have produced effective inhibitors that have entered or are entering the clinic, p38MAPK and IκB kinase 2 (IKK2). This in part is due to the fact that the majority of molecules discovered signalling from TNFR and TLR4/CD14 (LPS receptor) have involved protein/protein interactions, which as mentioned above are not easily amenable to inhibition by small molecules. In addition, notable lessons have been learnt about the requirement for appropriate models to study signalling; this is most dramatically demonstrated by experiences with NF-κB-inducing kinase (NIK). This kinase was identified as key factor in the TNF activation of NF-κB as a TRADD associated kinase. Expression of this kinase was found to be a powerful activator of NF-κB. Additional studies further indicated that NIK was important to numerous signalling pathways controlling NF-κB activation and potentially TNF production, suggesting that this kinase would be a key target for inflammation. However, these studies were mainly performed in transformed cell lines. When studies were performed in primary cells of human or murine origin, it was found that NIK was much more restricted, being confined to a limited numbers of receptors but not required for TNF production or function in normal cells or those derived from RA synovium (fig 1A) and sarcoïd (fig 1B). These data demonstrate a clear problem that it may not be possible to extrapolate signalling mechanisms from transformed cell lines, where they may be aberrant, to primary human tissue.

Our own studies on disease tissue have also demonstrated another aspect previously unappreciated, that although TNF is key to the pathogenicity of many diseases, the signalling molecules involved in its expression may vary between different diseases, presumably as a consequence of the stimulus involved. Figure 2 shows that although TNF is produced by both cells derived from RA synovium or sarcoïd BAL, the use of a dominant negative inhibitor of IκK2 resulted in significant inhibition of TNF production in the sarcoïd but not the RA tissue. Thus, it is quite possible that even though TNF may be a general target, a small molecular weight inhibitor of a given kinase may have a more restricted therapeutic profile.

Another aspect that needs to be considered is whether the function of the target when inhibited can be supplemented by another molecule, most likely a member of the same family.

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**Figure 1** NIK is essential for constitutive TNF production by primary rheumatoid synoviocytes (A) and sarcoïd bronchoalveolar lavage (BAL) (B). Dissociated rheumatoid synoviocytes or cells from a BAL were uninfected or infected with an empty adenovirus (Ad0) or adenoviruses expressing NIK (AdNIK), or the kinase dead version of NIK (AdNIKkd); (multiplicity of infection (moi)=100 (A); moi=150 (B)). Cell supernatants were collected after 24 hours, and secreted TNF levels were determined by enzyme linked immunosorbent assay (ELISA). Data represent one experiment and are representative of three independent experiments using separate donors (SEM).
Such functional redundancy is seen, for instance, with members of the tec family of tyrosine kinases.25

PROBLEMS OF SELECTIVITY OF TARGET AND DRUG

The last aspect to be considered here is the therapeutic index of any potential drug. The toxicity of a drug is normally of two types: non-mechanism based and mechanism based. Non-mechanism based toxicity depends on the selectivity of a drug for its target compared with its effect on other cellular proteins. The problem of how to produce drugs absolutely specific for the intended targets is a constant and elusive one. Studies by the Cohen laboratory26,27 have shown that some previously specific kinase inhibitors may be quite promiscuous when presented to enough kinases. This observation is perhaps not so surprising because there is a degree of conservation between kinase domains. As absolute selectivity may or may not be achievable the best that can be hoped for is that inhibition of “collateral” targets will not present enough of a problem to prevent the use of the drug. There also needs to be an awareness that screening of, for example, a kinase inhibitor against other kinases may not provide the full range of potential alternative targets as there may be effects on other totally unrelated proteins in remote tissues. An interesting example of this is CNI 1493 that was originally developed as an inhibitor of the arginine transporter in macrophages, then shown to also inhibit the activation of mitogen activated protein (MAP) kinases,28,29 and then found to activate the vagus nerve.30 Interestingly, in this case all the mechanisms have potential anti-inflammatory activity.

The problem of mechanism based toxicity is more intractable, as it is possible potentially to design around the problem of unacceptable selectivity of a drug but not the multiple physiological roles of an enzyme. Any inhibitor of a given enzyme regardless of its profile for other targets will produce unwanted toxicity owing to the importance of the target in processes unrelated to the disease. The fact that signalling enzymes are often used by multiple systems suggests that there will be a major problem. Thus two of the most investigated targets for the generation of blockers of TNF at the moment, IKK2 and p38MAPK, may have problems. The deletion of either kinase from mice results in death of the embryo.31,32 However, it can be difficult to judge the relevance of murine embryo development to disease states in the mature adult human. The initial demonstration of the importance of p38MAPK to TNF production raised high hopes that this would be a more tractable target for blocking TNF expression.33 However, given the availability of inhibitors of p38MAPK many studies have implicated this enzyme in several biological systems other than the control of TNF expression. These observations may underlie the observation that despite being more than eight years since the first inhibitors of p38MAPK were described none have yet made it past phase II.

A possible strategy for dealing with such a problem might be to investigate signalling molecules either proximal or distal to the p38MAPK in a given signalling pathway that may have a different cellular expression profile from p38MAPK and therefore not present the same problems of unrelated functions. In the case of p38MAPK this might be provided by Bruton's tyrosine kinase (BTK), which has been shown to regulate p38MAPK25 and potentially TNF production;34 unlike p38MAPK it has a much more restricted tissue expression and is found mainly in B cells, myeloid cells, and mast cells. There an inhibitor of BTK may have a more restricted mechanistic toxicity profile than an inhibitor of p38MAPK. Thus searching up and down the pathway may provide a strategy for dealing with mechanism based toxicity.

SUMMARY

The advent of anti-TNF biological agents has been a massive advance in our treatment of RA and other inflammatory diseases. However, it is acknowledged that there are major drawbacks, the greatest being cost. There is, therefore, clearly a massive market for small molecular weight inhibitors that would achieve similar effects to those of the biological agents. However, the effectiveness and safety of the biological agents has raised a very large hurdle that will need to be overcome if the potential cost benefit of such an inhibitor is to be realised.

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