DOI: 10.1002/cmdc.200600050

Inhibition of TNF-α Signaling: Divide and Conquer

Thorsten Berg*[a]

The cytokine tumor necrosis factor alpha (TNF-α) plays an important role in the inflammatory response to tissue injury and various viral and bacterial infections.[1] TNF-α forms homotrimers that bind to TNF-α receptor 1 (TNFR1) and TNF-α receptor 2, (TNFR2) inducing receptor trimerization, which in turn triggers the biological responses. As aberrantly increased TNF-α activity may lead to tissue damage, inhibitors of TNF-α are of clinical interest for the treatment of autoimmune diseases such as rheumatoid arthritis, psoriatic arthritis, Crohn's disease, and asthma. Clinical inhibition of aberrantly high levels of TNF-α is currently achieved by using a soluble form of TNFR2 coupled to the invariant part of immunoglobulins (Etanercept by Amgen and Wyeth Pharmaceuticals) or with anti-TNF-α antibodies (Infliximab by Centocor and Schering–Plough, and Adalimumab by Abbott). These protein-based agents have validated TNF-α as a therapeutic target and have yielded significant advances in the treatment of rheumatoid arthritis. Small-molecule inhibitors of TNF-α–TNF-α receptor binding may be beneficial, as they could be taken orally, thus eliminating the need for injection. Furthermore, the production costs of a small molecule are likely to be significantly lower than those for a protein-based agent, resulting in a cheaper drug.

A research group at Sunesis Pharmaceuticals has identified a small molecule that inhibits the interaction between TNF-α and TNFR1 in the micromolar concentration range (Figure 1a).[2] The substance is composed of a trifluoromethylphenyl indole and a dimethyl chromone moiety, which are linked by a dimethylamine spacer. X-ray crystallographic analysis revealed that the compound displaces one of the three TNF-α subunits and forms a complex with the remaining two subunits (Figure 1b). The symmetry of the resulting TNF-α–TNF-α–inhibitor complex closely resembles that of the homotrimeric complex (Figure 1c). The interaction between the inhibitor and the two TNF-α subunits appears to be largely hydrophobic and shape-driven, as no intermolecular hydrogen bonds or salt bridges were observed. Interestingly, 6 of the 16 amino acids of the remaining two TNF-α molecules with which the inhibitor interacts are tyrosine residues. Since the TNF-α–TNF-α–inhibitor complex is unable to bind to and stimulate TNF-α receptors, it effectively inhibits TNF-α signaling.

Surprisingly, the inhibitor did not simply bind to the TNF-α homotrimer and thereby block residues crucial for binding to its receptors, but instead displaced one TNF-α subunit. This led the authors to study the mechanism by which subunit displacement occurred. Two mechanisms are possible: either the compound binds to a TNF-α dimer only after one of the three TNF-α subunits has spontaneously dissociated (predissociation-dependent mechanism) or it binds to the intact TNF-α trimer and actively displaces one of the three TNF-α subunits (predissociation-independent mechanism). Thorough biochemical analysis revealed that the predissociation-independent mechanism was the case because binding of the substance to the intact TNF-α trimer could be detected, and the inhibitor accelerated the dissociation of one of the three TNF-α subunits by 600-fold. It is unclear how the compound dissociates the trimer, as the amino acids to which the inhibitor binds are buried in the intact trimer. The authors speculate that the molecule might contact its binding site by transient structural fluctuations. Another question is that of specificity: whereas the authors demonstrate specific inhibition of TNF-α-mediated degradation of the protein inhibitor of NF-κB (IκB) in one cellular assay, it would be interesting to learn how the interactions between other multimeric cytokines and their receptors are influenced by the inhibitor. Furthermore, it will be important to observe whether the substance has a beneficial effect in inflammatory disease model systems.

The identification of this inhibitor demonstrates that the function of a homotrimeric protein can be inhibited by displacement of one of the protein subunits by a small molecule. Moreover, the identification of the predissociation-independent mechanism of action demonstrates that small molecules can actively dissociate preformed protein complexes. Interestingly, the current study is not the first report of a substance that disrupts TNF-α trimers. About a decade ago, suramin, a symmetrical polysulfonated urea derivative used for the treatment of trypanosomiasis and onchoceriasis, was found to inhibit receptor binding of TNF-α, at least in part, by accelerating the dissociation of trimeric TNF-α by fourfold at 37 °C, whereas no effect was observed at 4 and 20 °C.[3] In contrast to the TNF-α inhibitor featured herein, suramin was found to completely dissociate TNF-α trimers to monomers. The new TNF-α inhibitor appears to be significantly more potent than suramin; despite being tested at a 30-fold lower concentration than suramin, it causes a 600-fold acceleration of TNF-α subunit dissocia-

[a] Dr. T. Berg
Max Planck Institute of Biochemistry
Department of Molecular Biology
Am Klopferspitz 18, 82152 Martinsried
(Germany)
Fax: (+49) 89-8578-2454
E-mail: berg@biochem.mpg.de

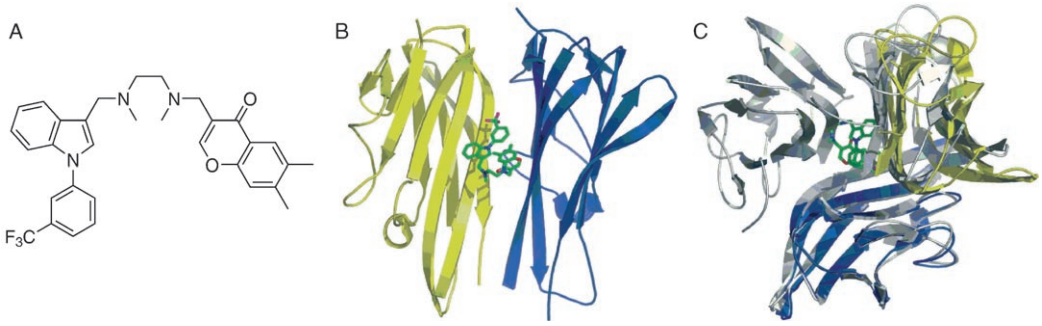

Figure 1. A) Structure of the TNF-α inhibitor. B) Crystal structure of the complex between the inhibitor and two TNF-α subunits. C) Superposition of the TNF-α dimer (yellow/blue) bound to the inhibitor (green) with the TNF-α trimer structure (gray). Parts B) and C) are reprinted with permission from Ref. [2], copyright 2005 AAAS.

tion, even at room temperature, a condition under which suramin is inactive towards TNF-α. Other substances which have been reported to inhibit the interaction between TNF-α and its receptors include cyclic peptides consisting of D-amino acids[4] and exocyclic small peptidomimetics corresponding to three critical binding sites of TNFR1.[5] Substances containing an *N*-alkyl 5-arylalkylidene-2-thioxo-1,3-thiazolidin-4-one core were found to block binding of TNF-α to TNFR1 by binding reversibly to the receptor with micromolar affinity in the dark. With exposure to light, which is generally not a physiological condition, unfortunately, the inhibitors could subsequently modify the receptor covalently by a photochemical reaction, resulting in activities in the nanomolar concentration range in vitro.[6]

As small-molecule drugs that directly target receptor binding of TNF-α have not yet been developed, alternative approaches to regulate TNF-α activity with small molecules are currently being explored in the clinical setting. The underlying rationale of these methods is the inhibition of signaling pathways that activate TNF-α as only one of a number of effects, and for which small-molecule drugs may be available in the near future. As TNF-α expression is regulated in part by the p38 MAP kinase pathway, the suitability of small-molecule kinase inhibitors of p38 for the treatment of rheumatoid arthritis is currently being investigated in clinical trials. The inhibition of phosphodiesterase type 4 (PDE4), the enzyme that catalyzes the conversion of cAMP to AMP, leads to an increased activity of protein kinase A, which in turn inhibits the activity of transcription factors such as NF-κB. NF-κB activates the genes not only for TNF-α, but also many others, including those for other cytokines. PDE4 inhibitors are currently under clinical investigation for the treatment of inflammatory airway diseases caused by excess activity of TNF-α. The teratogenic drug thalidomide and some of its derivatives also decrease the levels of cytokines, including TNF-α, and are therefore currently under clinical investigation to assess their suitability as therapeutics for the treatment of inflammatory disorders and certain tumors.[7] These indirect approaches to the reduction of TNF-α signaling come with the drawback of an increased likelihood of side effects, as the actual target of inhibition has a broader signaling role. It makes more sense to adopt the most direct, and thereby selective, approach towards the inhibition of TNF-α, that is blocking its interaction with its receptors, preferably with small molecules. It may still be a long way to the development of such a drug, but the new TNF-α inhibitor[2] highlighted herein is a big step in the right direction.

Acknowledgements

I extend my thanks to Professor Dr. Axel Ullrich for supporting my research. Our work is funded in part by the BMBF (NGFN-2, grant no. 01GS0451) and the Wilhelm Sander-Foundation.

Keywords: biological activity · drug design · inhibitors · protein–protein interactions · receptors

[1] M. A. Palladino, F. R. Bahjat, E. A. Theodorakis, L. L. Moldawer, *Nat. Rev. Drug Discovery* **2003**, *2*, 736 – 746.
[2] M. M. He, A. Stroustrup Smith, J. D. Oslob, W. M. Flanagan, A. C. Braisted, A. Whitty, M. T. Cancilla, J. Wang, A. A. Lugovskoy, J. C. Yoburn, A. D. Fung, G. Farrington, J. K. Eldredge, E. S. Day, L. A. Cruz, T. G. Cachero, S. K. Miller, J. E. Friedman, I. C. Choong, B. C. Cunningham, *Science* **2005**, *310*, 1022 – 1025.
[3] R. Alzani, E. Cozzi, A. Corti, M. Temponi, D. Trizio, M. Gigli, V. Rizzo, *Biochemistry* **1995**, *34*, 6344 – 6350.
[4] M. Kruszynski, D. J. Shealy, A. O. Leone, G. A. Heavner, *Cytokine* **1999**, *11*, 37 – 44.
[5] W. Takasaki, Y. Kajino, K. Kajino, R. Murali, M. I. Greene, *Nat. Biotechnol.* **1997**, *15*, 1266 – 1270.
[6] P. H. Carter, P. A. Scherle, J. K. Muckelbauer, M. E. Voss, R. Q. Liu, L. A. Thompson, A. J. Tebben, K. A. Solomon, Y. C. Lo, Z. Li, P. Strzemienski, G. Yang, N. Falahatpisheh, M. Xu, Z. Wu, N. A. Farrow, K. Ramnarayan, J. Wang, D. Rideout, V. Yalamoori, P. Domaille, D. J. Underwood, J. M. Trzaskos, S. M. Friedman, R. C. Newton, C. P. Decicco, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 11 879 – 11 884.
[7] S. K. Teo, D. I. Stirling, J. B. Zeldis, *Drug Discovery Today* **2005**, *10*, 107 – 114.

Received: February 27, 2006
Published online on May 4, 2006

