Development of an Interleukin (IL) 6 Receptor Antagonist That Inhibits IL-6-dependent Growth of Human Myeloma Cells

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Summary

The pleiotropic cytokine interleukin 6 (IL-6) plays a role in the pathogenesis of various diseases, such as multiple myeloma, autoimmune and inflammatory diseases and osteoporosis. Therefore, specific inhibitors of IL-6 may have clinical applications. We previously succeeded in developing receptor antagonists of IL-6 that antagonized wild-type IL-6 activity on the human Epstein-Barr virus (EBV)-transformed B cell line CESS and the human hepatoma cell line HepG2. However, these proteins still had agonistic activity on the human myeloma cell line XG-1. We here report the construction of a novel mutant protein of IL-6 in which two different mutations are combined that individually disrupt the association of the IL-6Rα/IL-6 receptor (R)α complex with the signaltransducing "β" chain, gp130, but leave the binding of IL-6 to IL-6Rα intact. The resulting mutant protein (with substitutions of residues Gln160 to Glu, Thr163 to Pro, and replacement of human residues Lys42-Ala57 with the corresponding residues of mouse IL-6) was inactive on XG-1 cells and weakly antagonized wild-type IL-6 activity on these cells. By introducing two additional substitutions (Phe171Leu, Ser177Arg), the affinity of the mutant protein for IL-6Rα was increased fivefold, rendering it capable of completely inhibiting wild-type IL-6 activity on XG-1 cells. Moreover, this mutant also antagonized the activity of IL-6, but not that of leukemia inhibitory factor, oncostatin M, or GM-CSF on the human erythroleukemia cell line TF-1, demonstrating its specificity for IL-6. These data demonstrate the feasibility of developing specific IL-6R antagonists. The availability of such antagonists may offer an approach to specifically inhibit IL-6 activity in vivo.

Interleukin 6 (IL-6) is a pleiotropic cytokine that acts on a wide variety of cells, exerting cellular proliferation, growth inhibition, and specific gene expression, sometimes accompanied by cellular differentiation (1). IL-6 has been suggested to be involved in the pathogenesis of several diseases, including inflammatory and autoimmune disorders and lymphoid malignancies (1). Indeed, specific inhibition of IL-6 in vivo by administration of neutralizing anti-IL-6 mAbs had beneficial effects in multiple myeloma, rheumatoid arthritis, Castleman's disease, and in experimental models for sepsis (2–6).

The IL-6 signal is transduced after homodimerization of a nonligand–binding signal transducer, gp130 (IL-6Rβ), which becomes associated with an 80-kD IL-6–binding chain (IL-6Rα) in the presence of IL-6 (1). Gp130 also transduces signals delivered by oncostatin M (OM), leukemia inhibitory factor (LIF), ciliary neurotrophic factor, and IL-11, in addition to those of IL-6, explaining the functional redundancy of these cytokines (for review see 7).

We have previously identified two regions of IL-6, distinct from the IL-6Rα binding site, which are important for IL-6 biological activity (8, 9). Mutations in each of these regions have little effect on IL-6–IL-6Rα binding, but disrupt the association of the IL-6Rα/IL-6Rα complex with IL-6Rβ. For purpose of clarity these regions are here designated β1 and β2. The β1 region consists of residues Gln153-His165 of IL-6. Some IL-6.β1 mutant proteins with substitutions of Thr158 to Arg or Gln160 to Glu, combined with Thr163 to Pro (IL-6.W158R/T163P or IL-6.Q160E/T163P), can antagonize the biological activity of wild-type (wt) IL-6 on the human EBV-transformed B cell line CESS and the human hepatoma cell line HepG2 (8 and de Hon, F. D., E. ten Boekel, J. Herrman, C. Clement, M. Ehlers, T. Taga, K. Yasakawa, Y. Ohsugi, T. Kishimoto, S. Rose-John, et al., manuscript submitted for publication). The β2 region was identified by studying human/mouse chimeric proteins of IL-6 and is composed of residues Lys42-Ala57 (9). An IL-6.β2 mutant pro-
tein with residues Lys42-Ala57 of human IL-6 exchanged with the corresponding residues of mouse IL-6, had an ~1,000-fold reduced specific activity in various bioassays, but displayed no antagonistic activity (9).

Recently we found that the IL-6.β1 antagonist mutant proteins had residual agonistic activity on the human myeloma cell line XG-1. This activity could be inhibited with anti-gp130 mAb, but could not be eliminated by extensive mutagenesis of the β1 region, suggesting that other parts of IL-6 that are important for association with gp130, might be involved (de Hon, et al., manuscript submitted for publication). Although the β1 and β2 regions are closely spaced in the putative tertiary structure of IL-6 (9), experimental evidence suggested that they are functionally distinct (de Hon, et al., manuscript submitted for publication). Combining a β1 with a β2 region mutant might therefore result in an IL-6 variant devoid of activity on XG-1 cells and with antagonistic properties. To test this hypothesis, the mutations of the IL-6.β1 mutant protein Q160E/T163P have here been combined with those of the above described IL-6.β2 mutant protein and the biological activity and receptor binding characteristics of the resulting protein (IL-6.β1.2) were examined.

Materials and Methods

Cytokines. The wt IL-6 preparation used as a standard was mature recombinant human (rh) IL-6 (Ala1-Met185) purified from Escherichia coli BL21 (DE3) carrying the pet8c-hlIL-6 cDNA expression vector (8). E. coli-derived rhOM and rhLIF were obtained from PreproTech (Rockyhill, NJ), hGM-CSF was from Sandoz Ltd. (Basel, Switzerland).

Construction of Expression Vectors. The T7 promoter vector pRSETDSD was used for expression of IL-6 mutant proteins (a kind gift of T. Stoyan, Institut für Biochemie der Rheinisch Westfälischen Technischen Hochschule, Aachen, Germany). The construction of expression vectors encoding the IL-6.β1 mutant protein (IL-6.Q160E/T163P) and the IL-6.β2 mutant protein (a mutant in which human Lys42-Ala57 is exchanged with the corresponding mouse residues) has been described (8, 9). The vector pRSET-IL-6.β1 was constructed by ligating an Ncol-XbaI 6-cDNA fragment from pRSET-IL-6.β2 carrying the IL-6.β1 mutations into Ncol-XbaI-digested pRSET-IL-6.β1. To construct pRSET-IL-6.β1,2, Phe171Leu/Ser177Arg, and Ser177Arg substitutions was created with PCR technology using primer and poly-HRP streptavidin (poly HRP; Janssen Biochimica, Weers, Belgium) without further washing. After

Figure 1. Biological activity of IL-6.β1, -β2, and -β1.2 on the human myeloma cell line XG-1 and erythroleukemia cell line TF-1. XG-1 and TF-1 cells were cultured for 3 and 2 d, respectively, in the presence of wt IL-6, IL-6.β1, IL-6.β2, or IL-6.β1.2 at the indicated concentrations and the induced proliferation was measured by tritiated thymidine incorporation. Data points represent mean cpm of triplicate cultures. The SD was generally <10% of these values for the XG-1 assay and <25% for the TF-1 assay. One representative experiment out of three is shown.
warming with PT, bound poly HRP was finally detected with the last two constant region domains of human IgG1 (sIL-6RB-IgG1). Used with immobilized sIL-6Rc and a fusion protein consisting of the IL-6Rc-dependent association with IL-6RB, an ELISA was constructed. 3,3',5,5'-tetramethylbenzidine (TMB; Merck, Darmstadt, Germany) conjugate was subsequently detected after extensive washing with mouse mAb specific for human IgG (MH16-1ME; Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), without intermediate washing. Bound sIL-6RB-IgG was detected with a HRP-conjugated anti-human IgG. Control, a mutant protein with mutations in the IL-6Rc binding site (Arg180Ser, Ala181Arg, and a deletion of the COOH-terminal amino acid residues). The average values of duplicate measurements are shown of one representative experiment out of three.

To measure the capacity of the IL-6 mutant proteins to induce the IL-6Rα-dependent association with IL-6RB, an ELISA was used with immobilized sIL-6Rα and a fusion protein consisting of 605 amino acids of the extracellular domain of gp130 (including the 22-amino acid signal peptide), fused to the hinge region and the terminal amino acid residues. The resulting vector encoding this protein and its expression in COS-7 cells will be described elsewhere (Ebeling, S. B., manuscript in preparation). For the ELISA, microtiter plates were coated with mAb MT18, washed, and incubated with sIL-6Rα as described above. After washing, various concentrations of IL-6 mutant proteins together with culture supernatant from COS-7 cells transfected with the sIL-6RB-IgG1 expression vector were added for 2 h. After this incubation period, wells were emptied and bound sIL-6RB-IgG was detected with a HRP-conjugated mouse mAb specific for human IgG (MH16-1ME; Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), without intermediate washing. Bound conjugate was subsequently detected after extensive washing with PT, as described above.

Results

IL-6/61,2 Is Inactive on Human Myeloma and Erythroleukemia Cells. We tested the biological activity of the IL-6/61,2 mutant protein on the human myeloma cell line XG-1 and the human erythroleukemia cell line TF-1 (Fig. 1). In contrast to the IL-6/61 and IL-6/62 mutant proteins, which displayed an ~1,000-fold reduced specific activity in the assays, the IL-6/61,2 double mutant protein was completely devoid of activity on XG-1 and TF-1 cells.

IL-6/61,2 Binds to IL-6Rα But Not to IL-6Rβ. IL-6 can bind to a soluble form of the extracellular domain of IL-6Rα (sIL-6Rα) with similar affinity as to the transmembrane receptor (13). Also, in the presence of the soluble extracellular domain of IL-6Rβ (sIL-6Rβ), ternary complex formation between IL-6, sIL-6Rα, and sIL-6Rβ can be detected (14). We therefore employed ELISAs with sIL-6R components to investigate the receptor binding characteristics of the IL-6 mutant proteins. As shown before, the IL-6/61 and IL-6/62 mutant proteins had a similar affinity for sIL-6Rα as wt IL-6, whereas the interaction with sIL-6Rβ was severely impaired (Fig. 2; references 8, 9). The binding characteristics of the IL-6/61,2 mutant protein were similar to those of the individual mutants, with a small (fivefold) reduction in affinity for sIL-6Rα (Fig. 2). In contrast to the lack of reactivity of IL-6/61,2 with sIL-6Rβ, we reproducibly observed some residual reactivity of IL-6/61 and IL-6/62. This might explain why the individual mutant proteins retained activity on XG-1 and TF-1 cells, whereas the double mutant was inactive. Thus, despite of its ability to form a complex with IL-6Rα, the IL-6/61,2 protein is inactive in XG-1 and TF-1 assays because it is unable to associate with IL-6Rβ and induce dimerization thereof.

IL-6/61,2 and IL-6/61,2/LR Act as IL-6R Antagonists on XG-1 Cells. The binding properties of the IL-6/61,2 protein suggested that it might antagonize wt IL-6 activity on XG-1 and TF-1 assays. Indeed, the IL-6/61,2 protein inhibited wt IL-6 activity on XG-1 cells to ~40%, when tested at a concentration of 10 μg/ml (Fig. 3). To increase the antagonistic capacity of the IL-6/61,2 mutant protein we subsequently introduced two substitutions (Phe171Leu and Ser177Arg) in the COOH terminus of the molecule. Individually, these substitutions had been reported to increase the affinity for IL-6Rα by approximately two- to threefold (15, 16). The resulting mutant protein IL-6/61,2/LR dis
played a fivefold increased affinity of sIL-6Rα compared with IL-6,β1,2 (data not shown). This mutant protein completely inhibited wt IL-6 activity on XG-1 cells when used at a 10-fold molar excess (Fig. 3). The inhibition could be reversed by adding increasing concentrations of wt IL-6, demonstrating that antagonism by the mutant protein preparation was not due to an aspecific toxic effect (data not shown).

**IL-6,β1,2/LR Specifically Inhibits IL-6 Activity on TF-1 Cells.**

We next measured the capacity of the IL-6,β1,2/LR mutant protein to inhibit the proliferation of TF-1 cells induced by wt IL-6, LIF, OM, and hGM-CSF. Whereas the IL-6–induced proliferation was completely abrogated by the mutant protein, the proliferation induced by the other cytokines was unaffected (Fig. 4). Thus, the IL-6,β1,2/LR protein can completely and specifically antagonize the biological activity of wt IL-6 on XG-1 and TF-1 cells.

**Discussion.**

To elucidate the mechanism of IL-6–receptor interaction and to generate IL-6 variants with potential therapeutic value, we and others study the structure-function relationships of human IL-6. Three regions of IL-6 have now been identified that are involved in the IL-6Rα–dependent association of IL-6 with IL6Rβ: the β1 and β2 regions described here and a recently described third region (provisionally designated B3) that is composed of residues Tyr31 and Gly35 (17). Individually mutating the β1, but not the β2 region, renders partial receptor antagonists, which inhibit IL-6 activity on a human EBV-transformed B cell line (CESS) and on hepatoma cell line HepG2, but not on XG-1 and TF-1 cells (8, 9; and de Hon et al., manuscript submitted for publication). For a β1 mutant protein partial antagonism on the human hepatoma cell line Hep3B was shown (17). It is here demonstrated that combining β1 and β2 mutations results in an IL-6 variant that is completely inactive on both the human IL-6 responsive cell lines XG-1 and TF-1 and the cell lines CESS and HepG2 and can antagonize IL-6 activity on these cells (Fig. 1 and data not shown). It will be interesting to test whether combining the β3 mutations with the β1 and/or β2 mutations gives similar results.

It is at present unclear what the exact roles of the β regions are in IL-6–receptor activation. It is established that IL-6Rβ dimerization is required for signal transduction (18). Stahl and Yancopoulos (19) postulated a general model for IL-6–like cytokines, in which β chain dimerization is induced by a monomeric cytokine. In this model, IL-6 has three receptor-binding sites: an α chain binding site and two β chain binding sites, to which the α and β chains sequentially associate to form an active signal transduction complex. Recently, however, evidence was obtained in experiments with sIL-6Rα and sIL-6Rβ, that the active high affinity IL-6R might consist of a hexameric complex of two molecules of each IL-6, IL-6Rα, and IL-6Rβ (20). In this complex, each IL-6 molecule binds to an IL-6Rα molecule. Although IL-6 binding to IL-6Rα might induce a conformational change in this chain leading to β chain association, formation of the hexameric complex, contacts between the IL-6 molecules themselves and between each IL-6 and IL-6Rβ molecule might be required. The mutations in the IL-6,β1,3 regions have little or no effect on IL-6Rα binding (8, 9, 17, and Fig. 2). Moreover, the mutations in the β1 and β2 regions do not seem to affect the overall conformation of IL-6, because the mutant proteins also retain reactivity with conformation specific mAb and are all active in bioassays with mouse cells (data not shown). It seems likely, therefore, that they affect either dimerization of IL-6, or a direct association with IL-6Rβ. Further experiments are in progress to elucidate the roles of the β1,3 regions in IL-6–receptor interaction.

IL-6 has been implicated to play a causative role in a wide variety of diseases (for a recent review see 21). Accordingly, various strategies are currently explored to inhibit IL-6 activity in vivo, including neutralizing IL-6–specific mAb (2, 22) and anti-IL-6R mAb (22, 23). An IL-6R antagonist, might constitute an alternative, additional strategy to specifically inhibit in vivo IL-6 activity. Preferably, because of low immunoreactivity, a receptor antagonist should be a natural variant of IL-6, like the naturally occurring receptor antagonist of IL-1α/β, IL-1RA (24), or a small (synthetic) IL-6-inhibiting compound. However, no such specific inhibitors of IL-6 activity have been described to date. An advantage of using an IL-6R antagonist for therapy could be that it might not induce the accumulation of wt IL-6 in the circulation, as has been observed both in phase I trials with anti-IL-6 mAb and in animal studies (3, 5, 25). Also, an IL-6R antagonist targets both the membrane-bound as well as the soluble form of IL-6Rα. In contrast to the soluble forms of other cytokine receptors which often inhibit cytokine activity, sIL-6Rα can associate with and trigger signal transduction through IL-6Rβ, when complexed to IL-6 (13). Increased levels of sIL-6Rα have been detected in patients with
HIV infection (26) and with multiple myeloma (27) and have been suggested to contribute to the disease processes. From our experiments it can be deduced that the possible therapeutic use of an antagonistic IL-6 mutant protein will require high doses of the molecule. Depending on the assay, a 10³-10⁵-fold molar excess over wt IL-6 is needed for complete inhibition (8, see Figs. 3 and 4). These doses are comparable, however, to the therapeutic doses of IL-1RA (28). Whether it is possible to improve the antagonistic capacity of IL-6 mutant proteins by introduction of additional, yet unidentified, substitutions that increase the affinity for IL-6Rα, remains to be established.

In conclusion, by combining two mutations that reduce IL-6 signal transduction but not IL-6R binding, we created for the first time an IL-6 variant that is completely inactive on human myeloma cells. Increasing the affinity of this mutant protein for the IL-6Rα resulted in an effective IL-6R antagonist. The availability of such an antagonist may offer an additional approach to specifically inhibit IL-6 activity in vivo.

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References

1. Kishimoto, T., S. Akira, and T. Taga. 1992. Interleukin-6 and its receptor: a paradigm for cytokines. Science (Wash. DC). 258:593.
2. Klein, B., J. Wijdenes, X.G. Zhang, M. Jourdan, J.M. Boiron, J. Brochier, J. Liautard, M. Merlin, C. Clement, B. Morel-Fournier, et al. 1991. Murine anti-interleukin-6 monoclonal antibody therapy for a patient with plasma cell leukemia. Blood. 78:1198.
3. Wendling, D., E. Racadot, and J. Wijdenes. 1993. Treatment of severe rheumatoid arthritis by anti-interleukin 6 monoclonal antibody. J. Rheumatol. 20:259.
4. Beck, J.T., H. Su-Ming, J. Wijdenes, R. Bataille, B. Klein, D. Vesole, K. Hayden, S. Jagannath, and B. Barlogie. 1994. Alleviation of systemic manifestations of Castleman's disease by monoclonal anti-interleukin-6 antibody. N. Engl. J. Med. 330:602.
5. Heremans, H., C. Dillen, W. Put, J. Van Damme, and A. Billiau. 1992. Protective effect of anti-interleukin (IL)-6 antibody against endotoxin, associated with paradoxically increased IL-6 levels. Eur. J. Immunol. 22:2295.
6. van der Poll, T., M. Levi, C.E. Hack, H. ten Cate, S.J.H. van Deventer, A.J.M. Eerenberg, E.R. de Groot, J. Jansen, H. Galant, H.R. Büller, et al. 1994. Elimination of interleukin 6 attenuates coagulation activation in experimental endotoxemia in chimpanzees. J. Exp. Med. 179:1253.
7. Kishimoto, T., T. Taga, and S. Akira. 1994. Cytokine Signal Transduction. Cell. 76:253.
8. Brakenhoff, J.P.J., F.D. de Hon, V. Fontaine, E. ten Boekel, H. Schooltink, S. Rose-John, P.C. Heinrich, J. Content, and L.A. Aarden. 1994. Development of a human interleukin-6 receptor antagonist. J. Biol. Chem. 269:86.
9. Ehlers, M., J. Grötzinger, F.D. de Hon, J. Müllberg, J.P.J. Brakenhoff, J. Liu, A. Wollmer, and S. Rose-John. 1994. Identification of two novel regions of human IL-6 responsible for receptor binding and signal transduction. J. Immunol. 153:1744.
10. van Dam, M., J. Müllberg, H. Schooltink, T. Stoyan, J.P.J. Brakenhoff, L. Graeve, P.C. Heinrich, and S. Rose-John. 1993. Structure-function analysis of interleukin-6 utilizing human/murine chimeric molecules. J. Biol. Chem. 268:15285.
11. Hirata, Y., T. Taga, M. Hibi, N. Nakano, T. Hirano, and T. Kishimoto. 1989. Characterization of IL-6 receptor expression by monoclonal and polyclonal antibodies. J. Immunol. 145:2900.
12. Mackiewicz, A., H. Schooltink, P.C. Heinrich, and S. Rose-John. 1992. Complex of soluble human IL-6-receptor/IL-6 up-regulates expression of acute-phase proteins. J. Immunol. 149:2021.
13. Faga, T., M. Hibi, Y. Hirata, K. Yamasaki, K. Yasukawa, T. Matsuda, T. Hirano, and T. Kishimoto. 1989. Interleukin 6 (IL-6) triggers the association of its receptor (IL-6-R) with a possible signal transducer, gp130. Cell. 58:573.
14. Yasukawa, K., K. Futatsugi, T. Saito, H. Yawata, M. Narazaki, H. Suzuki, T. Taga, and T. Kishimoto. 1992. Association of recombinant soluble IL-6-signal transducer, gp130, with a com-
plex of IL-6 and soluble IL-6 receptor, and establishment of an ELISA for soluble gp130. *Immunol. Lett.* 31:123.

15. Leebeek, F.W.G., K. Kariya, M. Schwabe, and D.M. Fowlkes. 1992. Identification of a receptor binding site in the carboxyl terminus of human interleukin-6. *J. Biol. Chem.* 267:14832.

16. Savino, R., A. Lahm, M. Giorgio, A. Cabibbo, A. Tramontano, and G. Ciliberto. 1993. Saturation mutagenesis of the human interleukin 6 receptor-binding site: implications for its three-dimensional structure. *Proc. Natl. Acad. Sci. USA.* 90:4067.

17. Savino, R., A. Lahm, A.L. Salvati, L. Ciapponi, E. Sporeno, S. Altamura, G. Paonessa, C. Toniatti, and G. Ciliberto. 1994. Generation of interleukin-6 receptor antagonists by molecular-modeling guided mutagenesis of residues important for gp130 activation. *EMBO (Eur. Mol. Biol. Organ.)* J. 13:1357.

18. Murakami, M., M. Hibi, N. Nakagawa, T. Nakagawa, K. Yasukawa, K. Yamanishi, T. Taga, and T. Kishimoto. 1993. IL-6-induced homodimerization of gp130 and associated activation of a tyrosine kinase. *Science (Wash. DC).* 260:1808.

19. Stahl, N., and G.D. Yancopoulos. 1993. The alphas, betas, and kinases of cytokine receptor complexes. *Cell.* 74:587.

20. Ward, L.D., G.J. Howlett, G. Discolo, K. Yasukawa, A. Hammacher, R.L. Moritz, and R.J. Simpson. 1994. The high affinity interleukin-6 (IL-6) receptor is a hexameric complex consisting of two molecules of each IL-6, IL-6 receptor and gp-130. *J. Biol. Chem.* 269:23206.

21. Akira, S., T. Taga, and T. Kishimoto. 1993. Interleukin-6 in biology and medicine. *Adv. Immunol.* 54:1.

22. Vink, A., P. Coulie, G. Warner, J.-C. Renaud, M. Stevens, D. Donckers, and J. Van Snick. 1990. Mouse plasmacytoma growth in vivo: enhancement by interleukin 6 (IL-6) and inhibition by antibodies directed against IL-6 or its receptor. *J. Exp. Med.* 172:997.

23. Suzuki, H., K. Yasukawa, T. Saito, R. Goitsuka, A. Hasegawa, Y. Ohsugi, T. Taga, and T. Kishimoto. 1992. Anti-human interleukin-6 receptor antibody inhibits human myeloma growth in vivo. *Eur. J. Immunol.* 22:1989.

24. Hannum, C.H., C.J. Wilcox, W.P. Arend, F.G. Joslin, D.J. Dripps, P.L. Heimdal, L.G. Arnels, A. Sommer, S.P. Eisenberg, and R.C. Thompson. 1990. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature (Lond.)* 343:336.

25. Lu, Z.Y., J. Brochier, J. Wijdenes, H. Brailly, R. Bataille, and B. Klein. 1992. High amounts of circulating interleukin (IL)-6 in the form of monomeric immune complexes during anti-IL-6 therapy. Towards a new methodology for measuring overall cytokine production in human in vivo. *Eur. J. Immunol.* 22:2819.

26. Honda, M., S. Yamamoto, M. Cheng, K. Yasukawa, H. Suzuki, T. Saito, Y. Ohsugi, T. Tokunaga, and T. Kishimoto. 1992. Human soluble IL-6 receptor: its detection and enhanced release by HIV infection. *J. Immunol.* 148:2175.

27. Gaillard, J.-P., R. Bataille, H. Brailly, C. Zuber, K. Yasukawa, M. Attal, N. Maruo, T. Taga, T. Kishimoto, and B. Klein. 1993. Increased and highly stable levels of functional soluble interleukin-6 receptor in sera of patients with monoclonal gamopathy. *Eur. J. Immunol.* 23:820.

28. Fischer, E., M.A. Marano, K.J. Van Zee, C.S. Rock, A.S. Hawes, W.A. Thompson, L. DeForge, J.S. Kenney, D.G. Remick, D.C. Bloedow, et al. 1992. Interleukin-1 receptor blockade improves survival and hemodynamic performance in *Escherichia coli* septic shock, but fails to alter host responses to sublethal endotoxemia. *J. Clin. Invest.* 89:1551.