Neutalizing monoclonal antibodies specific for human interleukin-6 (IL-6) bind two distinct sites on the IL-6 protein (sites I and II). Their interference with IL-6 receptor binding suggested that site I is a receptor-binding site of IL-6, whereas site II is important for signal transduction. Mutagenesis of site II could therefore result in the isolation of IL-6 receptor antagonists. To test this hypothesis, a panel of IL-6 mutant proteins was constructed that did not bind to a site II-specific monoclonal antibody. One such site II mutant protein (with double substitution of Glu-160 with Glu and Thr-163 with Pro) was found to be an antagonist of IL-6. It was inactive on human CESS cells, weakly active on human HepG2 cells, but active on mouse B9 cells. It could specifically antagonize the activity of wild-type IL-6 on CESS and HepG2 cells. The binding affinity of this variant for the 80-kDa IL-6 receptor was similar to that of wild-type IL-6. High affinity binding to CESS cells, however, was abolished, suggesting that the mutant protein is inactive because the complex of the 80-kDa IL-6 receptor and the mutant protein cannot associate with the signal transducer gp130. The human IL-6 antagonist protein may be potentially useful as a therapeutic agent.

Interleukin-6 (IL-6) is a multifunctional cytokine playing a central role in host defense mechanisms (for reviews, see Refs. 1-3). IL-6 exerts its multiple activities through interaction with specific receptors on the surface of target cells (4, 5). The cDNAs for two receptor chains have been cloned and code for transmembrane glycoproteins of similar size with a NH2-terminal cytokine receptor domain (6-10). The 80-kDa IL-6 receptor (IL-6R) binds IL-6 with low affinity ($K_d \sim 1$ nm) without triggering a signal (11). The IL-6-80-kDa IL-6R complex subsequently associates with gp130, which then transduces the signal (7, 11). gp130 itself has no affinity for IL-6 in solution, but stabilizes the IL-6-80-kDa IL-6R complex on the membrane, resulting in high affinity binding of IL-6 ($K_d \sim 10$ pm) (7). Recently, it was found that gp130 is also a constituent of the receptors for leukemia inhibitory factor, oncostatin M, and ciliary neurotrophic factor (for recent review, see Ref. 14; Refs. 12 and 13).

In a variety of human inflammatory, autoimmune, and neoplastic diseases, abnormal IL-6 production is observed and has been suggested to play a role in the pathogenesis (reviewed in Refs. 3 and 15-17; Ref. 18). A causative role for IL-6 in the pathogenesis of multiple myeloma was indeed demonstrated by the observation that administration of an anti-IL-6 antibody to a patient with plasma cell leukemia could block myeloma cell proliferation in the bone marrow (19). Thus, inhibitors of IL-6 biological activity are useful to study its role in disease and could have broad therapeutic applications.

One strategy to neutralize IL-6 activity could be by inhibition of the ligand/receptor interaction with specific receptor antagonists. The feasibility of such an approach was recently demonstrated with a natural occurring receptor antagonist for interleukin-1 (for review, see Ref. 21; Ref. 20), which is currently being tested for utility in a variety of diseases (22). For IL-6, however, no natural receptor antagonist has been identified so far.

Previously, we have shown that neutralizing monoclonal antibodies (mAbs) to human IL-6 (hIL-6) recognize two distinct epitopes, designated sites I and II, on the hIL-6 molecule (23). We speculated that one of these sites might be involved in binding to the 80-kDa IL-6R, whereas the other might be involved in an interaction with gp130. According to the IL-6R model, IL-6 variants that bind normally to the 80-kDa IL-6R, but are somehow defective in inducing the interaction of the IL-6-80-kDa IL-6R complex with gp130, might be able to prevent heterodimerization of the receptor and function as receptor antagonists.

In this paper, we investigated the roles of sites I and II in IL-6/receptor interaction. We now show evidence for a role of site I in binding to the 80-kDa IL-6R and for site II in signal transduction because a biologically inactive hIL-6 mutant protein with mutations in site II could bind to the 80-kDa hIL-6R, but antagonized the biological activity of wild-type hIL-6. Binding experiments suggest that signal transduction cannot occur because the complex of the mutant protein and the 80-kDa IL-6R cannot associate with gp130. This is the first demonstration that it is possible to separate receptor binding from biological activity of hIL-6.

**MATERIALS AND METHODS**

**Antibodies and Cytokines**

The production and purification of the IL-6-specific mAbs have been described in detail (23). mAb B1 (LN1-73-10) was a kind gift of Dr. F. Di Padova (Sandoz Pharma, Preclinical Research, Basel, Switzerland). The purified wild-type rhIL-6 preparation used throughout these experiments as a standard is derived from *Escherichia coli* carrying the HGF7 plasmid (24). Purification of rhIL-6/HGF7 has been described (25). The specific activity of purified rhIL-6/HGF7 was determined at the mouse B9 assay as $10^6$ units/mg. Recombinant IFN-γ was a kind gift from Genentech (San Francisco).
Expression Vectors and Bacterial Strains

Construction of the expression vector pUK-IL-6 has been described (25). For expression of rhIL-6 or rhIL-6 mutant proteins with this vector, E. coli DH5α (Life Technologies, Inc.) was used as the host. The bacteriophage T7 promoter vector pET8c and expression strain E. coli BL21(DE3) (26) were a kind gift of Dr. G. Pruyne (Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands).

Expression Library Construction and Recombinant DNA and Sequencing Protocols

The vector pUK-IL-6 was used for construction of the library of rhIL-6 mutant proteins with randomly distributed substitutions of Gin-153-Thr-163. Construction of the library has been described in detail (27). Following transformation to E. coli DH5α, ~1000 colonies were obtained. DNA manipulation procedures were performed as described (23, 25). Nucleotide sequences of selected mutants (see below) were obtained with cDNA-derived oligonucleotide primers on double-stranded DNA by using the Sequenase kit (United States Biochemical Corp.).

Preparation of E. coli Extracts for Library Screening with mAbs

Four-hundred ampicillin-resistant colonies from the expression library were transferred onto wells of 96-flat-bottom microtiter plates (Nunc) containing 100 μl of 100 mM Tris buffer (pH 8.5), and purification by anion-exchange chromatography. The nucleotide sequences were determined for mutant proteins that bound to mAb8(II), but not to mAb16(II).

Preparation and Quantification of E. coli Extracts for Biological Activity Measurements

To measure the biological activity of the mutant proteins that bound to mAb8(II), but not to mAb16(II), overnight cultures of E. coli DH5α carrying the mutant constructs were diluted 1:50 in 250 ml of LC am medium and subsequently cultured to an absorbance at 550 nm of 1.5. Bacteria were harvested by centrifugation, resuspended in 5 ml of 0.1% Triton X-100, and lysed by sonication. To solubilize rhIL-6-containing inclusion bodies, SDS was subsequently added to 1%. After 1 h of incubation at room temperature, SDS-insoluble material was removed by centrifugation (13,000 x g for 15 min). The biological activity of this SDS-solubilized material was directly measured in the mouse B9 and CESS assays starting from a 1:1000 dilution. At this dilution, the SDS did not affect the bioassays used. The IL-6 variant protein concentration of these preparations was determined by a competitive inhibition radioimmunoassay with IL-6-specific mAb CLB.IL-6/7 coupled to Sepharose 4B (Pharmacia LKB, Uppsala) and 125I-rhIL-6 in the presence of 10% SDS. Unlabeled rhIL-6 served as a standard. 

Expression and Purification of Mutant Proteins T163P and Q160E,T163P from E. coli

The IL-6 cDNA inserts from the vectors pUK-IL-6 T163P and pUK-IL-6 Q160E,T163P were subcloned in the vector pET8c, and the plasmids were transformed to E. coli BL21(DE3) for expression (28). The rhIL-6 variants were subsequently purified essentially as described (30). Briefly, the proteins were prepared from inclusion bodies by extraction with 6 M guanidine HCl, renaturation by dialysis against 25 mM Tris (pH 8.5), and purification by anion-exchange chromatography. The preparations were free of contaminating E. coli-derived proteins as judged by SDS-polyacrylamide gel electrophoresis followed by Coomassie Blue or silver staining. In some of the preparations, two bands were observed corresponding to the full-length mature protein and an ~10-amino acid shorter IL-6-degradation product. This degradation product does not significantly change the biological activity of the receptor binding of the full-length product because it generally constituted only 10-20% of the preparations. Moreover, carboxy-terminal cleavage of only 5 amino acids of IL-6 reduces both the bioactivity and receptor binding affinity 1000-fold (23,3). For expression of rhIL-6 or rhIL-6 mutant proteins with this vector, E. coli DH5α (Life Technologies, Inc.) was used as the host. The bacteriophage T7 promoter vector pET8c and expression strain E. coli BL21(DE3) (26) were a kind gift of Dr. G. Pruyne (Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands).

IL-6 Bioassays

Mouse B9 Assay—The hybridoma growth factor activity of rhIL-6 and variants was measured in the mouse B9 assay as described (31).

CESS Assay—B cell stimulatory factor-2 activity of rhIL-6 variants was measured as described (32). IL-6-induced IgG1 production by the cells was subsequently measured in a sandwich enzyme-linked immunoassay assay using a mouse mAb specific for human IgG1 (MH16-1M; C.L.B.) with a human serum as a standard (H00-1234; C.L.B.). Enzyme-linked immunosorbent assay procedures were as described (33).

HepG2 Assay—The hepatocyte stimulating activity of rhIL-6 variants was assessed by measuring the induction of C1 esterase inhibitor production by HepG2 cells as described (33). Following culturing to confluence (5 x 106 cells in 0.5-ml wells (Costar) in Iscove's modified Dulbecco's medium supplemented with 5% fetal calf serum, 5 x 10-5 M β-mercaptoethanol, 100 IU of penicillin, 100 μg/ml streptomycin, and 20 μg/ml human transferrin (Behringerwerke, Marburg, Germany), HepG2 cells were washed twice and stimulated with serial dilutions of rhIL-6 or rhIL-6 mutant proteins for 48 h in the same medium in duplicate. In some experiments, cells were washed again after 24 h, and the cultures were continued for another 24 h in the presence of the same stimulus. This procedure results in a higher stimulation index. After the incubation period, C1 esterase inhibitor synthesis was subsequently measured by sandwich radioimmunoassay with anti-C1 esterase inhibitor mAb RII coupled to Sepharose 4B and 125I-labeled sheep polyclonal anti-C1 esterase inhibitor IgG with normal human plasma as a standard as described (34).

Binding Experiments

The inhibitory effect of the mAbs on IL-6 binding to CESS cells was measured by using metabolically 35S-labeled rhIL-6 as described (5,35). The inhibitory effect of rhIL-6 and of the rhIL-6 Q160E,T163P mutant protein on 125I-rhIL-6 binding to NIH-3T3 fibroblasts transfected with an 80-kDa IL-6 receptor expression vector was measured as described (36). Inhibition of binding of 125I-rhIL-6 to the soluble IL-6R was measured as follows. The extracellular ligand-binding domain of the 80-kDa IL-6R was expressed in NIH-3T3 fibroblasts as described (37). Culture supernatants of these cells were diluted 1:2 20 mg Tris-SC (pH 7.5), 140 mM NaCl, 5 mM EDTA, 1% Triton X-100, 2 mM methionine, 0.01% NaN3 and incubated with 5 x 103 dpm 125I-rhIL-6 in the absence or presence of increasing concentrations of the rhIL-6 or Q160E,T163P protein for 2 h at 4°C. 125I-rhIL-6-soluble hIL-6R complexes were immunoprecipitated using an 80-kDa receptor-specific antisem and protein A-Sepharose. Sepharose-bound radioactivity was subsequently measured with a gamma-counter (37).

For Scatchard analysis of wild-type rhIL-6 and Q160E,T163P binding to CESS cells, rhIL-6 purified from Chinese hamster ovary cells expressing hIL-6 (a kind gift from Dr. D. Fischer, Interpharm Laboratories, Nes-Ziona, Israel) and Q160E,T163P, purified as described above, were labeled with 125I-Bolton-Hunter reagent (4000 Ci/mmol, diiodinated; Amersham, Amersham, United Kingdom) essentially as described (4,38). Briefly, 5 μg of rhIL-6 or mutant in 50 μl of borate buffer (50 mM NaB3O3, 0.02% Tween 20 (pH 8.5)) was added to 500 μCi of the same stimulus with occasional mixing. Both reactions were stopped by addition of 100 μl of 5 mg/ml glycine in phosphate-buffered saline for 10 min. 125I-Labeled rhIL-6 or...
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IL-6 variant (ng/ml)

**A**

**B**

**Fig. 3.** Activity of purified rhIL-6 variants in different bioassays for human IL-6. Shown is induction by wild-type rhIL-6 (C), T163P (○), and Q160E,T163P (△) of IgG1 production by CESS cells (A) and C1 esterase inhibitor (C1 inh.) production by HepG2 cells (B). For each assay, one representative experiment is shown. Data points represent averages of three measurements. Assays were performed as described under "Materials and Methods."

portant for the mAb16 epitope, but this has yet to be confirmed by analyzing mutant proteins with single substitutions of these residues.

Some Site II Mutant Proteins Are Inactive on Human CESS Cells—The biological activity of crude extracts of the various mutant proteins was subsequently measured both in the mouse B9 assay and in IgG1 production by human CESS cells. All mutant proteins were biologically active in the mouse B9 assay. However, although very active in the mouse B9 assay, no activity could be detected in the rhIL-6,T163P and rhIL-6,Q160E,T163P mutant protein preparations on CESS cells (Fig. 2).

Selective Agonism of Q160E,T163P on Human Cells—To confirm the above observation, both IL-6 mutant proteins were purified and tested for biological activity on CESS cells and on a second IL-6-responsive cell line of human origin. Fig. 3 (A and B) shows representative dose-response curves of the mutant proteins in the two assays. In Table I, the specific activities of the mutant proteins in these assays are depicted, together with the specific activities in the mouse B9 assay. The T163P mutant protein was active in all assays, but with a lower specific activity than that of wild-type rhIL-6. As with the crude protein, the purified Q160E,T163P mutant protein again did not induce IgG1 synthesis by the CESS cells, not even when tested at a 10⁶-fold higher concentration than the wild-type protein (Fig. 3A). At these high concentrations, this variant protein caused a weak increase in the production of the acute-phase protein C1 esterase inhibitor by HepG2 cells. This weak response was characterized by a strongly reduced maximal response as compared to wild-type rhIL-6 (Fig. 3B). On mouse B9 cells, the specific activity of Q160E,T163P was 6–7-fold lower than that of rhIL-6 (Table I).

**Antagonism of IL-6 Action on CESS and HepG2 Cells by Q160E,T163P**—We subsequently tested whether these mutant proteins were able to antagonize the biological activity of wild-type rhIL-6. Fig. 4 (A and B) shows that the Q160E,T163P mutant protein completely inhibited the wild-type IL-6 activity on CESS and HepG2 cells. In these experiments, 50% inhibition of IL-6 activity in CESS and HepG2 assays was observed with ~50 ng/ml and 1 μg/ml Q160E,T163P respectively, corresponding to 20- and 200-fold the concentration of wild-type rhIL-6 used to stimulate the cells. No antagonistic activity of the T163P mutant protein could be detected (data not shown). The inhibitory effect of Q160E,T163P on IL-6 activity in both the CESS and HepG2 assays could be reversed by high concentrations of wild-type rhIL-6, suggesting that the inhibitory mechanism is competitive inhibition of IL-6 receptor binding by Q160E,T163P (data not shown).

**Specificity of Antagonism by Q160E,T163P**—The production of the acute-phase protein C1 esterase inhibitor by HepG2 cells can be increased in response to both IL-6 and IFN-γ via separate mechanisms (33). To demonstrate the specificity of inhibition by the double-mutant protein, we tested whether Q160E,T163P could inhibit IFN-γ-induced C1 esterase inhibitor synthesis by the HepG2 cells. Because IFN-γ is more potent than IL-6 in this assay, we tested the effect of Q160E,T163P over a concentration range of IFN-γ. No inhibitory effects of Q160E,T163P were observed at any IFN-γ concentration tested. An example is shown in Fig. 5, demonstrating that the C1 esterase inhibitor synthesis induced by 5 ng/ml wild-type rhIL-6 was inhibited to background levels, whereas that induced by 1 ng/ml IFN-γ was unimpaired.

**Binding to 80-kDa IL-6R by Q160E,T163P**—The fact that the mutant protein Q160E,T163P could still be recognized by site I-specific mAb8 and that it could antagonize wild-type IL-6 activity on CESS and HepG2 cells suggested that the 80-kDa IL-6R-binding site of the mutant protein was still intact. To test this hypothesis, binding of this variant to the 80-kDa IL-6R (to both the membrane-bound and -soluble forms) was measured. In Fig. 6A, the capacity of Q160E,T163P to inhibit binding of 125I-labeled IL-6 to NIH-3T3 fibroblasts transfected with an expression vector encoding the 80-kDa IL-6R (36) was compared to that of wild-type rhIL-6. Fig. 6B shows the effect of the mutant protein and wild-type rhIL-6 on binding of 125I-labeled IL-6 to the soluble IL-6R. The results indicate that the mutant protein Q160E,T163P was 3–4-fold less efficient than wild-type rhIL-6 in inhibiting binding of 125I-rhIL-6 to the 80-kDa IL-6R.

**No High Affinity Binding of Q160E,T163P to CESS Cells**—The above data suggest that Q160E,T163P is inactive on human cells because, although it can efficiently bind to the 80-kDa IL-6R, the complex of mutant and receptor is deficient in triggering signal transduction through gp130. To test

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**Table I**

| rhIL-6 variant | Mouse B9 | CESS | HepG2 |
|----------------|----------|------|-------|
| rhIL-6.HGF7    | 1.7      | 213  | 1.5 x 10³ |
| T163P          | 5        | 3.3 x 10⁴ | 9.1 x 10⁴ |
| Q160E,T163P    | 11       | >10⁶ | >10⁶  |

*EC₅₀ values (the concentration of IL-6 variant giving a half-maximal response in the assays), expressed in picograms/milliliter, were estimated graphically from dose-response curves of the purified proteins. Values represent averages of at least three experiments performed over a time span of several months. S.D. values varied from 14 to 90% of these values.*
Antagonism of hIl-6 activity by Q160E,T163P. A, CESS cells were incubated with increasing concentrations of Q160E,T163P in the absence (○) or presence (□) of 2 ng/ml wild-type rhIL-6. Data are expressed as means ± S.D. (n = 3). B, HepG2 cells were incubated for 48 h with increasing concentrations of Q160E,T163P with or without 5 ng/ml rhIL-6. Data points are means ± S.D. (n = 2). For both assays, one representative experiment of three is shown. CI inh., C1 esterase inhibitor.

Fig. 5. Q160E,T163P inhibits biological activity of hIl-6, but not of IFN-γ on HepG2 cells. HepG2 cells were incubated with or without rhIL-6 (5 ng/ml) or recombinant human IFN-γ (1 ng/ml) in the absence or presence of 9 μg/ml Q160E,T163P. One of two experiments is shown. Data are means ± S.D. (n = 2). C1 inh., C1 esterase inhibitor; med., medium; wt, wild-type.

whether Q160E,T163P could still bind with high affinity to human cells, we performed receptor binding experiments using 125I-labeled Q160E,T163P and CESS cells. Coulie et al. (5) and Taga et al. (4) detected a single class of binding sites on CESS cells with $K_D$ values of -30 pm (1700 sites/cell) and 340 pm (2700 sites/cell), respectively. However, both with E. coli-derived 125I-rhIL-6 (data not shown) and with Chinese hamster ovary cell-derived glycosylated rhIL-6, we always observed two binding sites. In Fig. 7, the results from representative experiments are displayed in Scatchard plots. Two binding sites were statistically significant for wild-type rhIL-6 (Fig. 7A; $F = 26.14$ ($p = 0.001$) for a one- versus two-site fit), with $K_D$ values of 13 pm (coefficient of variation (CV) = 40%, 430 sites/cell (CV = 30%)) and 360 pm (CV = 31%, 3200 sites/cell (CV = 9%)). Q160E,T163P, however, exhibited only a single class of binding sites, with a $K_D$ of 500 pm (Fig. 7B; CV = 59%, 7000 sites/cell (CV = 49%)). A two-site fit of the 125I-Q160E,T163P binding data was not statistically significant ($F = 0.03$ ($p = 0.9710$) for a one- versus two-site fit). From this experiment, we concluded that Q160E,T163P is inactive on CESS cells because the binding of the mutant-80-kDa IL-6R complex to gp130 cannot occur.

**DISCUSSION**

In this paper, we show for the first time that receptor binding and signal transduction of hIL-6 can be uncoupled. This is evidenced by the following observations. 1) The Q160E,T163P mutant protein was inactive on CESS cells (the responses of Q160E,T163P are within the standard deviation of the background control), yet antagonized the biological activity of wild-type rhIL-6 on these cells. 2) The antagonist protein had an affinity similar to that of wild-type rhIL-6 for the 80-kDa IL-6R. According to the model for IL-6/receptor interaction, high
A Human IL-6 Receptor Antagonist

Affinity binding of the IL-6-80-kDa IL-6R complex to gp130 is a prerequisite for IL-6 signal transduction (7, 11). Our results support this model because Q160E,T163P bound only with low affinity to CESS cells, suggesting that the complex of the mutant protein and the 80-kDa IL-6R could not associate with gp130 to trigger a signal. Because IL-6 does not bind to gp130 in the absence of the 80-kDa IL-6R, it is not clear whether there is a direct interaction between IL-6 and gp130 in the complete receptor complex (11). Recent evidence from cross-linking experiments suggests that IL-6 and gp130 are in very close proximity in the IL-6-receptor complex (42, 43). Without structural information from crystallographic studies on the ligand-receptor complex, however, it is difficult to prove the existence of a contact and the possible role of IL-6 residues Gin-169 and/or Thr-163 therein.

Selective Agonist of Q160E,T163P—On HepG2 cells, at high concentrations of the Q160E,T163P mutant protein, we reproducibly observed a small partial agonist activity, characterized by a maximal response of ~10–20% of that of wild-type IL-6 (Fig. 3B). This might indicate that the antagonist-80-kDa IL-6R complex still exhibits some affinity for gp130, which we did not detect in the binding experiments with CESS cells. For the recently described hIL-4 (44) and mouse IL-2 (45) antagonist variants, the response also varied between cell lines studied and could be explained by differences in sensitivity of the cell lines to the respective wild-type cytokines: in the more sensitive cell lines, full receptor occupancy was not required to elicit a maximal response. Partial agonist activity of a mutant that was inactive on insensitive cells was explained by occupancy of spare functional receptors on the very responsive cell types, compensating for the receptor activation defects of the mutants (44, 45). This explanation cannot be applied to our results, however, because CESS cells, which were nonresponsive to the Q160E,T163P mutant, were more sensitive to wild-type rhIL-6 than HepG2 cells: for HepG2 cells, half-maximal stimulation was achieved at 70 pm rhIL-6, whereas 10 pm induced half-maximal stimulation of CESS cells (Table I). Although we have as yet no explanation for this observation, our results might be due to differences in IL-6 receptors on CESS and HepG2 cells (43). In contrast to its activity on human cells, the Q160E,T163P mutant protein was nearly fully active on mouse cells, with a 6–7-fold reduced specific activity as compared to wild-type rhIL-6 in the mouse B9 assay. At first glance, this seemed to be due to the extremely high sensitivity of B9 cells to IL-6: half-maximal proliferation is induced by 0.08 PM, corresponding to a receptor occupancy of only 0.8%, assuming a high affinity $K_d$ for the mouse IL-6R of ~10 pm. However, when we tested the Q160E,T163P mutant protein on the insensitive mouse plasmacytoma cell line T1165, which requires 10 pm IL-6 for half-maximal activation, as with B9 cells, the specific activity was ~15% of that of wild-type rhIL-6. This suggests that in the mouse system, the interaction of the Q160E,T163P-80-kDa IL-6R complex with gp130 is not as strongly affected as in the human system and that the double mutant should still be able to bind with high affinity to mouse cells. Further experiments are in progress to resolve the above issues.

Localization of Gln-180 and Thr-193 in Putative Tertiary Structure of hIL-6—Unfortunately, the tertiary structure of IL-6 is unknown. Based on homology comparisons, however, IL-6 belongs to the large group of cytokines that have an antiparallel four-a-helical bundle core structure similar to that of growth hormone, which include granulocyte colony-stimulating factor, myelomonocytic growth factor, erythropoietin, and prolactin and also oncosenin M, leukemia inhibitory factor, and ciliary neurotrophic factor (8, 47, 48). For IL-6, the GH structure indeed seems to be a useful working model: Fig. 8 shows the localization of sites I and II in the hypothetical three-dimensional model for hIL-6 based on the GH structure, mAbB1(I) was shown to recognize residues at both the amino and carboxyl termini of IL-6, which fits with close proximity of helices A and D in the model (23). Site I and II-specific mAbs are capable of forming a sandwich with monomer rhIL-6 in enzyme-linked immunosorbent assays, which also agrees with the model (23). Site I on IL-6 and residues close to it are likely to be the 80-kDa receptor-binding site. 1) Site I-specific mAbs strongly inhibit receptor binding, and 2) site I colocalizes with Ser-175–Arg-185, which were recently shown to be essential for biological activity and receptor binding of IL-6 (23, 49–53). Site I also maps closely to a region essential to bioactivity in the NH2-terminal α-helix (Ile-80–Asp-35) (25, 29). This region on hIL-6 partially colocalizes with binding site 1 on human GH,
which was identified as a patch consisting of three discontinuous segments: the loop between GH residues 54 and 74 (the A-B loop), the COOH-terminal half of helix D, and, to a lesser extent, the NH2-terminal region of helix A (54, 55). Whether the A-B loop in hIL-6 is also part of the 80-kDa binding site is as yet unknown.

Gln-160 and Thr-163 are located in the C-D loop and at the beginning of helix D, respectively, and are part of site II. This region does not colocalize with site 2 on human GH, which was identified as a patch consisting of three discontinuous regions of these cytokines on biological activity and gp130 interaction.

Conclusion—in this report, we described for the first time the isolation of an IL-6 analog that could antagonize the activity of wild-type hIL-6 in some in vitro bioassays. The dose range in which this molecule inhibited IL-6 activities in these bioassays is similar to that reported for the natural occurring IL-1ra (59). The pleiotropy of IL-6 and the broad distribution of its receptors make selective therapeutic application of receptor antagonists difficult to envision. However, the observation that it is possible to isolate an IL-6 variant that is active in some (but not all) bioassays may point to the possibility of isolating selective agonists of IL-6 that retain useful activities, but antagonize IL-6 activity where desired. Otherwise, combination of an IL-6 antagonist with other cytokines that share some (but not all) of the functions of IL-6 might in part restore the useful activities of IL-6. Detailed information concerning the in vivo sites of production and the activities of IL-6 and cytokines with similar activities is required before considering these options. Development of IL-6 inhibitor strategies seems very appropriate regarding the speed with which diseases are discovered in which IL-6 seems to play a role (60, 61).

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