Measurement of Whole Body Interleukin-6 (IL-6) Production: Prediction of the Efficacy of Anti–IL-6 Treatments

By Zhao Yang Lu, Hervé Brailly, John Wijdenes, Régis Bataille, Jean-François Rossi, and Bernard Klein

A major limitation on the therapeutic use of cytokine antagonists is that the amount of cytokine to be neutralized in vivo is not presently known. We previously reported that anti-interleukin-6 (IL-6) monoclonal antibody (MoAb) administered to a patient with multiple myeloma (MM) induced high amounts of IL-6 to circulate in the form of monomeric immune complexes. Based on this observation, the present study developed a new methodology to estimate daily IL-6 production in 13 patients with MM or renal cancer who received anti-IL-6 MoAb. Treatment was considered effective when the production of C-reactive protein (CRP) was inhibited. The production of this acute-phase protein by hepatocytes is dependent on the activation of IL-6 gp130 transducer. Inhibition of tumor proliferation was also evaluated in patients with MM. In 7 of 13 patients whose CRP production was completely inhibited (>96%) and who showed some antitumor effects, whole-body IL-6 production in vivo was less than 18 μg/d (median, 5.7 μg/d; range, 0.5 to 17.5 μg/d). In the other 6 patients, subtotal inhibition of CRP production and a lack of antitumor response were associated with high IL-6 production (median, 180 μg/d; range, 18 to 358 μg/d). These in vivo observations were consistent with mathematical modeling that predicted that anti–IL-6 MoAb treatment would be efficient only in low IL-6 producers. These data indicate the difficulty of neutralizing IL-6 with a single anti–IL-6 MoAb in vivo and call for new strategies to avoid accumulation of IL-6 in the form of stable immune complexes.

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The involvement of cytokines in various tumoral, autoimmune, and inflammatory diseases has led to the development of cytokine-binding proteins (CBP), eg, monoclonal antibodies (MoAbs) to cytokines or soluble cytokine receptors, to neutralize cytokine activity in vivo. Clinical trials with anti–interleukin-6 (IL-6) MoAb have been initiated in patients with multiple myeloma (MM), rheumatoid arthritis, Castleman’s disease, human immunodeficiency virus lymphoma, or renal carcinoma (RC). Anti-tumor necrosis factor (TNF) MoAbs have been used in the treatment of patients suffering from hairy cell leukemia or gram-negative septicemic shock. Other CBP, such as soluble IL-1 receptor, soluble TNF receptor, or immunoadhesins derived therefrom, have also been developed for clinical use in humans.

A major difficulty in the evaluation of the efficacy of cytokine antagonists in vivo is a lack of data about whole-body production of a cytokine under normal and pathologic conditions. Such information is required to predict the amount of CBP needed to neutralize the target cytokine in vivo. Indeed, several groups have reported that CBP can increase cytokine half-life and induce the circulation of large amounts of cytokine in the form of complexes. In a patient with MM treated with anti-IL-6 MoAb, the half-life of IL-6 in the form of IL-6/anti-IL-6 complexes was increased 200-fold. This finding was confirmed in animals treated with anti–IL-6 MoAb, anti–IL-3 MoAb, anti–IL-4 MoAb, or soluble IL-4 receptor.

In the case of high cytokine production in vivo, the concentration of circulating CBP may not be sufficient to prevent the disruption of cytokine/CBP complexes by the cell membrane high-affinity cytokine receptor. Several groups have suggested that CBP in such cases behaves as a cytokine agonist in vivo. Once again, knowledge of whole-body cytokine production in a patient to be treated with CBP might help prevent such adverse effects.

In the present study, this CBP cytokine carrier effect was used to estimate daily whole-body production of IL-6 in 13 patients with MM or RC treated with murine anti–IL-6 MoAb. We found that low or high IL-6 production in vivo was strictly correlated with the clinical efficacy of anti–IL-6 treatment as determined by inhibition of C-reactive protein (CRP) production or tumor proliferation in patients with MM. The production of this acute-phase protein by human hepatocytes is induced by the different cytokines activating the gp130 IL-6 transducer. In vivo production was also strongly inhibited in various patients treated with anti–IL-6 MoAb.

Our methodology is of general relevance to the evaluation of CBP treatments involving MoAbs and soluble receptors. Within 2 days after the beginning of treatment, it is possible to determine whether cytokine overproduction is a limiting factor and thus avoid inefficient therapy.

MATERIALS AND METHODS

Patients. After informed consent was obtained, anti–IL-6 therapy was administered to 6 patients with MM, extramedullary proliferation, and resistance to chemotherapy and to 7 patients with metastatic RC resistant to IL-2 immunotherapy. Patients were treated with B-E8 anti–IL-6 MoAb (IgG) prepared by J. Wijdenes (Diachone, Besançon, France), which was administered in a 1-hour infusion every day at 8 AM for periods of 10 to 60 days. Patients with RC received a daily dose of 20 mg of B-E8 MoAb throughout the treatment. These doses were doubled (40 mg/day) on day 13 for patient DE and on day 15 for patient AT because of incomplete CRP inhibition. Five patients with MM received a daily dose of 20 mg and 1 patient (MU) received a daily dose of 8 mg. For patient

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CH, the MoAb dose was reduced to 10 mg/d on day 3 of treatment and was increased to 20 mg on day 11. Neutrophil counts for this patient decreased on day 3 due to toxic antibiotic therapy and then increased, again once this therapy was discontinued on day 8. The clinical results for these anti-IL-6 MoAb treatments have been reported elsewhere. Briefly, complete blockage of tumor proliferation occurred in 2 patients (TE and MU) with MM. In these patients, the serum concentration of monoclonal Ig was decreased by 50% and 30% respectively. No antitumoral response was noted in patients with RC. Serum CRP concentrations (monitored daily) became undetectable in 5 patients with RC (FA, AT, GO, BO, and CA) and in 2 with MM (TE and MU). Immunization to anti-IL-6 murine MoAb was detected between days 7 and 15 of treatment in 9 of 13 patients.

All immunized patients produced anti-idiotypic antibodies specific for B-E8 MoAb. Two patients (SO and BO) also developed antibodies directed at the murine IgG1 isotype. In these 2 patients, B-E8 MoAb cleared rapidly from the circulation and treatment efficacy was lost. In patients who developed only anti-idiotypic antibodies, serum levels of B-E8 MoAb were unchanged and CRP production remained inhibited, indicating that treatment efficacy was not affected by the presence of human antibodies to idiotypic determinants of B-E8 MoAb.

Reagents. Recombinant IL-6 was provided by D. Stinchcomb (Synergen, Boulder, CO); anti-IL-6 B-E4 and B-E8 MoAb were provided by J. Wijdenes; and anti-IL-6 AH-65 MoAb was provided by H. Brailly (Immunotech, Marseille, France). These three antibodies bind to distinct epitopes. Natural IL-6 was obtained from H. Brailly (Immunotech, Marseille, France). These three antibodies directed at the murine IgG1 isotype. In these 2 patients, B-E8 MoAb cleared rapidly from the circulation and treatment efficacy was lost. In patients who developed only anti-idiotypic antibodies, serum levels of B-E8 MoAb were unchanged and CRP production remained inhibited, indicating that treatment efficacy was not affected by the presence of human antibodies to idiotypic determinants of B-E8 MoAb.

B9 hybridoma assay. Serum IL-6-like bioactivity was evaluated using the B9 hybridoma bioassay. Twenty-five thousand B9 cells were cultured with serial dilutions of the sample in 96-well, flat-bottomed culture dishes in 100 µL of RPMI 1640 medium containing 5% FCS. After an incubation period of 4 days at 37°C in 5% CO2, the cells were exposed to MTT (Sigma, St Louis, MO) for 4 h at 37°C, as previously described. One unit of B9-stimulating activity (B9-SA), defined as the amount inducing half-maximal optical densities, corresponded to approximately 5 pg of recombinant IL-6.

Affinity chromatography. Affinity columns (protein A-Sepharose, B-E4-Sepharose, B-E8-Sepharose, and anti-IgG(mouse)-Sepharose) were prepared by coupling protein A (Sigma), B-E4 MoAb, B-E8 MoAb, or rat antimouse IgG antibody (Nordic Immunology, Amsterdam, The Netherlands) to cyanogen bromide-activated Sepharose 4B (Pharmacia, Uppsala, Sweden). The columns were used as previously described. The bound material was eluted at acidic pH (0.1 mol/L glycine-HCL, pH 2.4) according to the manufacturer's recommendations, immediately neutralized with 1 mol/L NaHCO3, and then assayed for B9-SA at different dilutions.

Gel filtration. A 4-ml sample was loaded onto a Sephadex G-200 column (100 cm × 2.6 cm) and chromatographed at 4°C with 50 mmol/L phosphate-buffered saline (PBS; pH 7.2) as eluting buffer; the flow rate was 12 mL/h and the fraction volume 4 mL. All fractions were assayed for B9-SA at different concentrations. Purified human IgG, bovine serum albumin (Sigma), ovalbumin, and equine myoglobin (Sigma) were used as standard molecules for estimating the molecular weight of B9-SA. To dissociate expected immune complexes, the samples were dialyzed for 12 h with a 0.1 mol/L glycine-HCl buffer (pH 2.3) and then gel-filtrated with the same acid buffer. Each fraction was neutralized with 1 mol/L NaHCO3 and assayed for B9-SA at different concentrations.

Determination of plasma IL-6 and plasma B-E8 MoAb concentrations. To determine the plasma IL-6 concentration bound to B-E8 anti-IL-6 MoAb, we used the two anti-IL-6 MoAbs (AH-65 and B-E4) that recognize different epitopes on the IL-6 molecule than those recognized by B-E8 MoAb. AH-65 was used as a catcher and B-E4 as a tracer. This methodology was previously described in detail. Detection of circulating free IL-6 in the patient (ie, not bound to B-E8 MoAb) was performed using an AH-65/B-E8 enzyme-linked immunosorbent assay (ELISA). B-E8 MoAb concentration was assayed using an ELISA, as previously described.

Calculation of daily IL-6 production. On the basis of the mathematical procedure developed below (equation 5), daily determinations of circulating IL-6 (in the form of monomeric immune complexes) and anti-IL-6 MoAb were performed to estimate the overall daily production of IL-6 induced to circulate by anti-IL-6 MoAb.

This mathematical procedure required the verification of two assumptions, ie, that the half-life of the immune complexes was similar to that of free MoAb and that no elimination route existed for IL-6 other than immune complexes. The first assumption was likely for monomeric immune complexes, as previously shown in animal models, and was confirmed by monitoring the accumulation kinetics of anti-IL-6 MoAb and IL-6-anti-IL-6 MoAb monomeric complexes at the beginning of treatment. As indicated below (see Fig 3), these accumulation curves were parallel, yielding a common half-life of 3 to 4 days. With respect to the second assumption, the two major elimination routes for cytokines in vivo are consumption by cells and renal elimination. When complete blockage of CRP production in vivo by anti-IL-6 MoAb occurred, it may reasonably be assumed that anti-IL-6 MoAb prevented IL-6 binding to cell surface receptors and elimination by renal filtration. Thus, all IL-6 produced in vivo was induced to accumulate in the form of monomeric immune complexes, so that daily overall IL-6 production should have been close to the value estimated by the mathematical procedure below. When CRP inhibition was partial, it may be concluded that overall IL-6 production in vivo was higher than the estimated value.

Mathematical procedure for calculating overall daily IL-6 production. Let [MoAb] represent the concentration of circulating MoAb at the beginning of day , just before the injection of anti-IL-6 MoAb on day, ; TiR = the half-life of circulating MoAb and b = e−b/(300); MoAb = the dose of MoAb at the beginning of day, and V = the volume of injected MoAb diffusion in the body.

Hence, [MoAb] = MoAb/V; [MoAb] = b[MoAb] + MoAb/b; [MoAb] = b[MoAb] + b[MoAb] + b[MoAb] + b[MoAb] + b[MoAb] + b[MoAb] (equation 1). Thus, [MoAb] = (bV)(MoAb) + bMoAb + bMoAb + bMoAb + bMoAb + bMoAb. When the dose of injected MoAb is constant, MoAb = MoAb = MoAb = MoAb = MoAb. Thus, [MoAb] = (MoAb/V) (1 + b + b + b + b + b) (equation 2).

The concentration of circulating MoAb converges to [MoAb] = (MoAb/V)(b−1−b) (equation 3).

Equations 2 and 3 can be used to fit the experimental curve of circulating MoAb and to provide experimental data for b. Hence IL-6 equals the overall production of IL-6 trapped by anti-IL-6 MoAb during day, and induced to circulate in the form of immune complexes; V = the volume of injected MoAb diffusion in the body; [IL-6] = the concentration of circulating IL-6 in the form of monomeric immune complexes at the beginning of day, = just before injection of anti-IL-6 MoAb on day, ; TIL-6 = the half-life of circulating IL-6 in the form of monomeric immune complexes; and b = e−b/(300). Thus, [IL-6] = [IL-6] + [IL-6] (1 + b)/2V (equation 4).

For monomeric immune complexes, the half-life is similar to that of free MoAb, so that b = b, which is the case for IL-6/B-E8 MoAb monomeric immune complexes (see above and Fig 1).

By combining equations 1 and 4, IL-6, can be determined by the equation: [IL-6] = 2 MoAb[b[IL-6] − b[IL-6],]/(2MoAb − b[MoAb] + b[MoAb] + b[MoAb]) (1 + b) (equation 5).

Numerical modeling of B-E8 MoAb ability to neutralize IL-6 binding to its high-affinity receptor. Our numerical modeling procedure is based on data in the literature describing the interactions of IL-6 with the two chains of its membrane receptor and the soluble forms...
EFFICACY OF ANTI-IL-6 TREATMENTS

The plasma sample and the culture supernatant were dialyzed for 12 hours before being applied to the Sephadex G-200 column with an 0.1 mol/L glycine-HCl eluting buffer (pH 2.3). The flow rate was 12 mL/h, and 4 mL fractions were collected. Each fraction was neutralized with 1 mol/L NaHCO₃ and assayed for B9 cell stimulating activity (B9-SA).

of these two chains. This procedure depends on a novel approach for the numerical resolution of complex multimolecular equilibria. In this model, IL-6 first binds the membrane IL-6 receptor (IL-6R) or its soluble form (sIL-6R). IL-6/IL-6R or IL-6/sIL-6R complexes then bind the gp130 transducer chain. These complexes may also bind the soluble form of gp130 (sgp130), which behaves as an antagonist. B-E8 anti-IL-6 MoAb blocks the binding of IL-6 to IL-6R or sIL-6R. Numerical modeling predicts the fraction of gp130 transducer activated by the IL-6/IL-6R or IL-6/sIL-6R complexes as a function of IL-6 and anti-IL-6 MoAb concentrations. The affinities of the interactions of the different components were estimated from data in the literature or determined by ELISA for assaying IL-6 complexed to B-E8 MoAb or free IL-6. Figure 2A shows that an ELISA using AH-65 and B-E4 anti-IL-6 MoAb recognized different epitopes on the IL-6 molecule than those recognized by B-E8 MoAb. Free IL-6 as well as preformed complexes of IL-6 and B-E8 MoAb diluted in human plasma were detected (sensitivity, approximately 30 pg/mL of IL-6). No activity was detected when IL-6 was complexed with B-E4 MoAb. Conversely, AH-65/B-E8 ELISA detected free IL-6 as well as IL-6/B-E4 complexes, but not IL-6/B-E8 complexes (sensitivity, approximately 30 pg/mL of IL-6; Fig 2B).

IL-6/B-E8 monomeric immune complexes have the same half-life as free B-E8 MoAb in vivo. Figure 3 displays the accumulation kinetics of B-E8 MoAb in patients treated with anti-IL-6 MoAb (Fig 3A, patients with MM; Fig 3C, patients with RC). A plateau generally obtained after 6 to 8 days of treatment indicated a 3- to 4-day half-life for the MoAb. In 2 cases (patient SO with MM and patient BO with RC), a decrease in plasma MoAb concentrations was observed after, respectively, 7 and 13 days of treatment due to immunization against B-E8 MoAb and subsequent clearance of this MoAb. In patient DE (RC), an increase in B-E8 MoAb concentrations occurred on day 13 when injected doses of B-E8 were doubled (Fig 3C). In patient CH (MM), the variations in B-E8 MoAb concentrations reflected the injection course. When an AH-65/B-E4 ELISA was used (results not shown), a high IL-6 concentration was detected in the plasma of every patient treated with B-E8 MoAb. This activity was entirely bound to B-E8 MoAb, as shown by the gel filtration experiments described above (Fig 1) and the absence of a signal when AH-65/B-E8 ELISA was used (results not shown). The accumulation kinetics of IL-6/B-E8 MoAb complexes are indicated in Fig 3B and D. For 10 patients, these curves paralleled those obtained for B-E8 MoAb, showing that IL-6/B-E8 MoAb half-life in vivo was similar to that of B-E8 MoAb (ie, 3 to 4 days). In patient CH (MM), there was a threefold decrease in the concentration of circulating IL-6, in association with severe neutropenia due to toxic antibiotic therapy.

ELISA for assaying IL-6 complexed to B-E8 MoAb or free IL-6. Figure 2A shows that an ELISA using AH-65 and B-E4 anti-IL-6 MoAb recognized different epitopes on the IL-6 molecule than those recognized by B-E8 MoAb. Free IL-6 as well as preformed complexes of IL-6 and B-E8 MoAb diluted in human plasma were detected (sensitivity, approximately 30 pg/mL of IL-6). No activity was detected when IL-6 was complexed with B-E4 MoAb. Conversely, AH-65/B-E8 ELISA detected free IL-6 as well as IL-6/B-E4 complexes, but not IL-6/B-E8 complexes (sensitivity, approximately 30 pg/mL of IL-6; Fig 2B).

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RESULTS

IL-6 accumulation in the form of monomeric immune complexes during anti-IL-6 MoAb treatment. In agreement with previous reports, a high activity stimulating B9 hybridoma growth (B9-SA) was found in the plasma of all patients at the beginning and after discontinuance of anti-IL-6 treatment and in some cases throughout the treatment period (results not shown). Using a methodology previously reported by us, we found that this activity consisted of IL-6 bound to the murine anti-IL-6 MoAb. In fact, IL-6 was retained on protein A or antimurine Ig Sepharose columns, indicating that it was linked to the murine MoAb (results not shown). It was eluted at peaks of 25 and 180 kD, respectively, after gel filtration with or without acidic conditions (Fig 1). Finally, 25 kD activity was inhibited by an anti-IL-6 MoAb (results not shown).

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Predictions of the efficacy of anti–IL-6 treatment. We investigated whether the efficacy of anti–IL-6 treatment was limited by IL-6 production. Table 1 shows the mean concentrations of circulating B-E8 anti–IL-6 MoAb and IL-6 from day 7 to 12 of treatment, at which time a steady state was achieved (Fig 3), as well as the ratio of these concentrations. Patients were classified according to the latter parameter. The mean inhibition of CRP production from days 7 to 12 and the mean IL-6 production values from day 2 to 12 of treatment are also given (Table 1). As indicated in Table 1, CRP production was completely inhibited (>96%) only in patients producing less than 18 μg of IL-6 per day for whom the ratio of IL-6 to anti–IL-6 MoAb molar concentrations was less than $4 \times 10^{-3}$. The mathematical procedure described in the Materials and Methods enabled us to plot theoretical curves for the fraction of gp130 transducer bound to IL-6/sIL-6R complexes for IL-6 concentrations of 0.1 to 1 μmol/L (ie, 2.5 to 25,000 ng/mL) and MoAb concentrations of 20 to 200 nmol/L (ie, 3 to 30 μg/mL). Interestingly, the fraction of bound gp130 in the presence of anti–IL-6 MoAb depended only on the IL-6/anti–IL-6 MoAb molar ratio, at least within the selected concentration ranges (Fig 5). Moreover, 10-fold variations in the concentrations of different components used in numerical modeling did not affect the theoretical curve when an sIL-6R concentration of 2 nmol/L was used. This sIL-6R value is the physiologic concentration found in plasma of patients with MM or RC.\textsuperscript{34} Numerical analysis was in very good agreement with the observed inhibitions of CRP production and IL-6/anti–IL-6 MoAb molar ratios in patients, as shown in Fig 5, which plots the observed inhibitions of CRP production over the theoretical curve. This analysis confirmed that there was a critical level in IL-6 production (18 μg/d) or the IL-6/anti–IL-6 MoAb molar ratio (4 \times 10^{-3}). Above this critical level, it was predictable that anti–IL-6 MoAb would no longer be efficient in blocking IL-6 activity, which is consistent with the lack of complete inhibition of CRP production in 8 patients (Fig 5 and Table 1).

It is noteworthy that the ratios of IL-6 to anti–IL-6 MoAb molar concentrations on day 2 of treatment were close to the mean ratios calculated when a steady state was achieved (Table 1). This finding is not surprising because daily IL-6 production was stable throughout treatment and the kinetics of IL-6 and anti–IL-6 MoAb accumulation were parallel (Figs 3 and 4). Thus, for each patient, the doses of anti–IL-6 MoAb required to neutralize IL-6 could be predicted as early as day 2 of treatment.

DISCUSSION

This report considers the ability of an MoAb to IL-6 to neutralize this cytokine in patients with MM or RC in vivo. IL-6 produced in vivo may exist in free form or be bound to the extracellular matrix\textsuperscript{35} or to plasma proteins.\textsuperscript{36} It can be eliminated by binding to cell surface receptors and internalization,\textsuperscript{37} renal filtration, or degradation by proteases (this last route has not been shown in vivo). Injection of an anti–IL-6 MoAb disturbs this distribution pattern. If the antibody is in sufficient concentration and has a high affinity with IL-6, it will bind free IL-6 and disrupt IL-6 binding to plasma...
proteins\textsuperscript{11} and probably to the extracellular matrix. In addition, anti–IL-6 MoAb will block IL-6 binding to cell surface receptors, and the high-molecular-weight IL-6/anti–IL-6 complex will no longer be eliminated through the kidneys.

By blocking the different IL-6 elimination routes, anti–IL-6 MoAb can thus accumulate large amounts of IL-6 circulating in the form of monomeric complexes. We initially described this phenomenon in humans 2 years ago,\textsuperscript{16} and it was recently confirmed by several groups in animal models using anti–IL-6 MoAb\textsuperscript{11,12} or other CBP.\textsuperscript{13,14} In this study, the concentration of circulating IL-6 ranged from 630 to 77,880 pg/mL in the 13 patients during treatment (range before treatment, 0 to 56 pg/mL). This accumulation of circulating IL-6 in the form of immune complexes was due to their long half-life (ie, 3 to 4 days), which is at least 200-fold higher than that of free IL-6.\textsuperscript{37} This finding is in agreement with those of previous reports concerning the half-life of monomeric immune complexes of hapten-anti-hapten antibodies in animal models.\textsuperscript{24}

This accumulation of IL-6 in the form of stable immune complexes is a serious limitation on treatments with anti–IL-6 MoAb. If IL-6 production is too high, the ratio of IL-6 to free MoAb molar concentrations will also be high and the high-affinity IL-6 receptor will disrupt IL-6/anti–IL-6 immune complexes, as recently discussed.\textsuperscript{16,17} To deal with this complex situation, we developed a numerical model taking into account the various chains of membrane IL-6 receptors and their soluble forms (IL-6R, gp130, sIL-6R, and sgp130). This model shows that the IL-6/anti–IL-6 ratio should be at least lower than 4 molecules of IL-6 to 1,000 molecules of anti–IL-6 MoAb, which was achieved in 7 patients in whom complete inhibition of CRP production was observed in vivo. In vitro, the production of this acute-phase protein by hepatocytes is induced by activation of gp130 transducer, which is achieved mainly by IL-6\textsuperscript{38} but also by other gp130-activating cytokines, ie, ciliary neurotrophic factor, IL-11, leukemia inhibitory factor, or oncostatin M.\textsuperscript{18} Our results here show that there was no evidence
that these other gp130-activating cytokines are involved in CRP production in the patients studied. In fact, CRP production was completely inhibited in the 7 patients whose IL-6 activity was predicted to be fully inhibited by anti-IL-6 MoAb. These data confirm the relevance of using CRP production to monitor the efficacy of anti-IL-6 treatment. Moreover, antitumoral effects were observed only in the patients with MM in this group. For patient MU, a complete inhibition of tumor cell proliferation was found throughout the 2-month treatment. For patient TE, a 50% reduction in monoclonal Ig concentration was found within 1 month of treatment. In the other 6 patients with MM, this ratio was too high (up to 25-fold), allowing only partial inhibition of CRP production in vivo and providing no antitumoral effects (Table 1). To model the neutralizing capacity of anti-IL-6 MoAb, we assumed that local concentrations of IL-6, anti-IL-6 MoAb, sIL-6R, and sgp130 were close to their plasma concentrations. This was true for hepatic tissue because IL-6 was produced in distant tissues (mainly at tumor sites) in these cancer patients. IL-6 circulated through the blood, arriving in the liver in the form of immune complexes that had the same molecular weight and probably the same diffusion ability as free antibody. This explains why the theoretical predictions for inhibition of gp130 transducer activation by anti-IL-6 MoAb fitted well with observed inhibitions of CRP in vivo. In tumor sites in which IL-6 is produced and thus found at higher concentrations than in plasma, the MoAb concentrations required to neutralize IL-6 should be higher than those in plasma. In these circumstances, it is easy to understand why a patient whose CRP production was only partially inhibited had no chance of responding to treatment.

This study emphasizes the difficulty of neutralizing IL-6 by means of a single anti-IL-6 MoAb in vivo, even though...
EFFICACY OF ANTI-IL-6 TREATMENTS

Table 1. Biologic Parameters of Patients Treated With Anti-IL-6 MoAbs

<table>
<thead>
<tr>
<th>Patients</th>
<th>Antitumoral Response</th>
<th>Anti-IL-6 MoAb Concentration (µg/mL)</th>
<th>IL-6 Concentration (ng/mL)</th>
<th>Ratio of IL-6 to Anti-IL-6 MoAb Molar Concentration (×10^3)</th>
<th>Ratio of IL-6 to Anti-IL-6 MoAb Molar Concentration on Day 2 (×10^3)</th>
<th>Observed Inhibition of CRP (%)</th>
<th>Daily IL-6 Production (µg/d)</th>
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<td>TE (MM) Yes</td>
<td>15.31</td>
<td>1.51</td>
<td>0.59</td>
<td>0.72</td>
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<tr>
<td>AT (RC) No</td>
<td>18.44</td>
<td>3.96</td>
<td>1.29</td>
<td>1.74</td>
<td>97</td>
<td>7.9</td>
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<tr>
<td>GO (RC) No</td>
<td>20.16</td>
<td>5.46</td>
<td>1.62</td>
<td>1.41</td>
<td>100</td>
<td>5.7</td>
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<tr>
<td>MU (MM) Yes</td>
<td>2.99</td>
<td>1.28</td>
<td>2.57</td>
<td>2.00</td>
<td>100</td>
<td>4.1</td>
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<tr>
<td>BO (RC) No</td>
<td>13.00</td>
<td>7.21</td>
<td>3.30</td>
<td>2.24</td>
<td>98</td>
<td>17.5</td>
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<tr>
<td>CA (RC) No</td>
<td>20.47</td>
<td>12.44</td>
<td>3.65</td>
<td>1.30</td>
<td>96.5</td>
<td>14.7</td>
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<tr>
<td>Partial inhibition of CRP</td>
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<tr>
<td>SE (RC) No</td>
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<td>4.50</td>
<td>5.86</td>
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<tr>
<td>SO (MM) No</td>
<td>6.70*</td>
<td>11.89*</td>
<td>10.65</td>
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<td>88</td>
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<td>CH (MM) No</td>
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<td>35.03</td>
<td>35.38</td>
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<tr>
<td>DE (RC) No</td>
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<td>60.75</td>
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<td>BU (MM) No</td>
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<td>56.61</td>
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<tr>
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<td>94.40</td>
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<td>60</td>
<td>&gt;358.4</td>
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Concentrations of anti-IL-6 MoAb and IL-6 and observed inhibition of CRP are the mean values from days 7 to 12 of treatment when a steady state was achieved, except for 2 patients (see notes). Daily IL-6 productions are the mean values for the first 12 days of treatment.

* Value of mean of days 4 to 8 because of subsequent immunization.
† Mean of values of days 4 to 8 because of subsequent severe neutropenia.
‡ Mean of values of days 2 to 4.

the B-E8 anti-IL-6 MoAb used had a strong affinity with IL-6 (kd, 10^-11 mol/L) and was one of the most potent neutralizing anti-IL-6 MoAbs in our hands. Our injected doses of MoAb (20 mg/d) were already high but should have been at least 25-fold greater to neutralize IL-6 in high IL-6-producing patients. Such predicted doses are far too elevated for therapeutic use. A new strategy could consist in injecting several anti-IL-6 MoAbs that recognize different epitopes on the IL-6 molecule. We have shown in an animal model that the resulting polymeric complexes in this case are cleared within 1 hour. Thus, we are currently investigating this new therapeutic approach in the hope of considerably reducing the half-life of these immune complexes in humans.

Our findings in these patients treated with anti-IL-6 MoAb might be of general use for antagonizing cytokines in vivo. Several antagonists are now being developed using either MoAb or soluble cytokine receptors linked or not to Fc fragments of Ig. Recent discussions and demonstrations in animal models relative to soluble IL-4 receptor or various MoAbs to IL-3, IL-4, IL-6, or IL-7 suggest that these CBP will be able to protect the cytokine from its classical elimination routes, allowing it to accumulate in the form of complexes. The efficacy of treatment with these CBP will depend on the ratio of cytokine to free CBP and on total cytokine production in vivo. Thus, it is important to have an estimation of whole-body cytokine production before CBP injection, which is precisely the objective of the original methodology described here. For a monomeric cytokine, it is conceivable to inject a humanized anticytokine antibody for 2 days. Our results here indicate that the measurements of circulating anticytokine antibody and cytokine on day 2 will allow an estimation of whole-body cytokine production predictive of the minimal CBP doses required to neutralize the cytokine in vivo.
REFERENCES


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Measurement of whole body interleukin-6 (IL-6) production: prediction of the efficacy of anti-IL-6 treatments

ZY Lu, H Brailly, J Wijdenes, R Bataille, JF Rossi and B Klein