Murine Anti-Interleukin-6 Monoclonal Antibody Therapy for a Patient With Plasma Cell Leukemia


A patient with primary plasma cell leukemia resistant to chemotherapy was treated for 2 months with daily intravenous injections of anti-interleukin-6 (IL-6) monoclonal antibodies (MoAbs). The patient's clinical status improved throughout the treatment and no major side effects were observed. Serial monitoring showed blockage of the myeloma cell proliferation in the bone marrow (from 4.5% to 0% myeloma cells in the S-phase in vivo) as well as reduction in the serum calcium, serum monoclonal IgG, and the serum C-reactive protein levels. The serum calcium and serum monoclonal IgG corrected by approximately 30%, whereas the C-reactive protein corrected to undetectable levels during treatment. No major side effects developed, although both platelet and circulating neutrophil counts decreased during anti-IL-6 therapy. A transient immunization was detected 15 days after the initiation of the treatment, which could explain the recovery of myeloma cell proliferation after 2 months of treatment (2% myeloma cells in the S phase). In conclusion, this first anti-IL-6 clinical trial demonstrated the feasibility of injecting anti-IL-6 MoAbs, and also a transient tumor cytostasis and a reduction in IL-6-related toxicities. It gave insight into the major biologic activities of IL-6 in vivo and may serve as a basis for further development of anti-IL-6 therapy in myeloma and other IL-6-related diseases.

INTERLEUKIN-6 (IL-6) is a potent growth factor in vitro for fresh tumor cells in human multiple myeloma (MM),1,2 which makes it possible to obtain myeloma cell lines whose proliferation is completely dependent on exogenous IL-6.3 Recent observations of our group favor an involvement of IL-6 in the proliferation of myeloma cells in vivo, especially in patients with terminal disease. We have shown increased serum IL-6 levels in patients with terminal disease, especially those with plasma cell leukemia (PCL), as compared to those with stable disease.6 In addition, we have also shown that the best responsiveness of myeloma cells to IL-6 in vitro was observed in patients with proliferating myeloma cells in vivo.7 These results prompted us to evaluate the therapeutic effect of anti-IL-6 monoclonal antibodies (MoAbs) in a patient with fulminant primary PCL with a high myeloma cell proliferation in vivo. The aim of our study was to carefully evaluate the feasibility of injecting anti-IL-6 MoAbs and also evaluate the possibility of blocking myeloma cell proliferation in vivo. We report here an inhibition of the myeloma cell proliferation together with a blockage of the IL-6 bioactivity for 1½ months in the patient treated with anti-IL-6 MoAbs, without the occurrence of major side effects. In addition, this study provides important insight into several biologic activities of IL-6 in vivo.

PATIENT, MATERIALS, AND METHODS

Case Report

A 61-year-old male patient presented primary PCL, extensive bone lesions, hypercalcemia, renal deficiency, anemia, leukocytosis, massive bone marrow invasion by malignant plasma cells, and 25% myeloma cells in the peripheral blood. The patient had 8.41 g/dL monoclonal IgG in the serum without light chains in the urine. A slight improvement was obtained with symptomatic treatments and 2 VAD regimens. After the second VAD regimen, a fulminant progression was observed with identical symptoms to those observed at diagnosis and the patient was treated with murine anti-IL-6 MoAbs as described below.

At day 0 of anti-IL-6 treatment, features presented by the patient were the following: performance status (ie, Karnofsky scale) 10%; hemoglobin level 8.5 g/dL; white blood cells 7,700 Clmm3 including 12% plasmablasts; serum calcium level 3.75 mmol/L; serum albumin level 2.49 g/dL; serum C-reactive protein (CRP) level 70 mg/L; IgG monoclonal Ig 8.3 g/dL and serum β2-microglobulin 4.8 mg/L. On days 41 through 44, 49 through 52, and 56 through 59, dexamethasone (40 mg/d intravenously) was injected with the rationale of limiting the effects of a putative immunization. At the end of the treatment with anti-IL-6 MoAbs, two additional VAD regimens were administered but the patient died of infection 1½ months later.

Injection of the Anti-IL-6 Antibodies

The antibodies were used in vivo after informed consent was obtained from the patient, the local ethical committee, and the head of the university hospital in agreement with the French laws in force at the time of the clinical trial (January 1990). Two anti-IL-6 MoAbs, termed B-E4 and B-E8, were successively administered following the schedule outlined in Table 1. In the following, the first day of anti-IL-6 injection is termed day 0. The xth day before treatment and the yth day of treatment are termed day x and day y, respectively. The anti-IL-6 MoAbs were administered as a 1-hour infusion (at 8 AM) in 100 mL physiologic serum with 0.5% human serum albumin. The first infusion was given 24 hours after a negative skin test (made with the anti-IL-6 MoAbs). The doses of anti-IL-6 MoAbs were chosen to reach serum MoAb concentrations similar to those known to inhibit the
Table 1. Inhibition of the In Vitro Spontaneous Proliferation of Bone Marrow Myeloma Cells by Anti-IL-6 Antibodies

<table>
<thead>
<tr>
<th>% Myeloma Cells</th>
<th>Labeling Index</th>
<th>Control</th>
<th>Anti-IL-6</th>
<th>Anti-IL-6 + IL-6</th>
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</thead>
<tbody>
<tr>
<td>Day – 36†</td>
<td>65</td>
<td>1.7</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Day 0</td>
<td>93</td>
<td>4.5</td>
<td>8</td>
<td>0.3</td>
</tr>
<tr>
<td>Day 10</td>
<td>64</td>
<td>0</td>
<td>10</td>
<td>0.5</td>
</tr>
<tr>
<td>Day 22</td>
<td>61</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Day 45</td>
<td>58</td>
<td>0</td>
<td>7.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Day 60</td>
<td>65</td>
<td>2.5</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

Bone marrow cells were cultured for 5 days with culture medium alone or with 10 μg/mL B-E8 anti-IL-6 MoAb or with 10 μg B-E8 MoAb and 50 ng/mL rlIL-6. At the end of the culture process, the percentages of myeloma cells in the S phase were determined.

†Days before or after beginning the treatment.

Materials and Methods

Collection of peripheral blood cells and serum samples. Peripheral blood or bone marrow was collected at 7 AM, 1 hour before B-E4 or B-E8 MoAb injections. Peripheral blood serum and plasma were stored at –20°C until required. Peripheral blood or bone marrow mononuclear cells were obtained by centrifugation of heparinized samples over Ficoll-Hypaque gradients.

Proliferation and culture assays of myeloma cells. The percentages of myeloma cells were determined by cytoplastic immunofluorescence using anti-κ light chain antibodies directly coupled to fluorescein (Kallestadt, Austin, TX). The percentages of plasma cells in the S phase were determined using an anti-bromodeoxyuridine MoAb (Immunotech, Marseilles, France) and a rhodamine-labeled goat antimouse Ig (Jackson Laboratories, West Grove, PA) in a double-fluorescence technique described elsewhere. At least 500 to 5,000 myeloma cells were examined to determine the percentages of plasma cells in the S phase. This examination ensured that the lowest percentages could be determined with 30% precision and a P value <0.05.

Cells were cultured at 10^5 cells/mL in RPMI 1640 culture medium supplemented with 5 x 10^-3 mol/L 2-mercaptoethanol (2-ME) and 5% fetal calf serum for 5 days. This supplemented RPMI 1640 culture medium was used for all cultures and was termed “culture medium.” In some groups, the anti-IL-6 (B-E8, 10 μg/mL) or control purified murine serum IgG (Sigma Chemicals, St Louis, MO) were added at the initiation of the cultures. To reverse the effect of the anti-IL-6 MoAb, the MoAb was incubated with human IL-6 (50 ng/mL) for 2 hours before being added to the cultures. On day 5, the percentage of myeloma cells and the percentage of myeloma cells in the S phase were determined as described previously.

Evaluation of circulating anti-IL-6 MoAb levels. The levels of circulating anti-IL-6 MoAbs were determined by an enzyme-linked immunosorbent assay (ELISA) specifically detecting mouse IgGs. Polystyrene plates (Immuno I; Nunc, Copenhagen, Denmark) were coated with purified goat IgG against mouse Ig (10 μg/mL in phosphate-buffered saline [PBS]) at room temperature. Plates were then saturated with 1% bovine milk proteins (BMP) in PBS for 1 hour. After five washings in PBS containing 0.05% Tween 20 (Sigma Chemicals), 100 μL of the patient’s sera diluted in PBS-BMP-Tween (1% BMP in PBS containing 0.05% Tween 20) were added for 1 hour at room temperature. Plates were washed five times with PBS-BMP-Tween before a solution containing peroxidase-conjugated goat antimouse Ig light chains (Tago Inc, Burlingame, CA) was added for 1 hour, and then washed again another five times. The reactivity was determined by the intensity of the enzymatic reaction to the substrate O-phenylenediamine measured by absorbance at 492 nm (Titertek Multiskan MC; Flow, Irvine, Scotland) after addition of 50 μL 2N H2SO4. Mouse Ig concentrations were calculated by using a reference curve obtained with serial dilutions of B-E4 or B-E8 MoAbs.

Detection of human antibodies to anti-IL-6 MoAbs. The presence of human antibodies to anti-IL-6 MoAbs was checked by ELISA. Plates (Immuno I; Nunc) were coated with either 10 μg/mL (in PBS overnight at room temperature) B-E4 MoAb, B-E8, or a control MoAb of the same isotype as either B-E4 (anti-herpes virus as IgG2b) or B-E8 (CD37, BL4 as IgG1). We checked that the plates were coated by the same amounts of each MoAb by using a peroxidase-conjugated goat antimouse Ig (Tago Inc). Plates were saturated with PBS-BMP and the patient’s serum samples (diluted 1/50 in PBS-BMP) were added for 1 hour at room temperature. The human Ig were detected by incubating plates with peroxidase-conjugated goat anti-human IgG or IgM (Jackson Laboratories).

Quantitation of serum proteins. Serum proteins were determined by rate immunonephelometry on the Beckman array protein system using Beckman monoclonal reagents (Beckman Instruments, Brea, CA). The following serum proteins were assayed: C-reactive protein (CRP), α1-antitrypsin (α1-AT), albumin, C3 and C4 complement components, IgG, IgA, and IgM.

IL-6 bioassay and source of IL-6. Biologic activity of IL-6 was evaluated using the B9 hybridoma bioassay (generous gift from L. Aarden, Amsterdam, The Netherlands). This bioassay is specific for human IL-6 and does not detect any other known human cytokines. One unit of IL-6 was defined as the amount yielding half-maximal proliferation and corresponded to about 1 pg rIL-6. In some experiments, the XG-1 human myeloma cell line was used with a protocol similar to that used for the B9 cells. Purified human rIL-6 was provided by N. Vita and P. Ferrara (Sanofi Elf Biorecherches, Labège, France).

RESULTS

Dependence on IL-6 of the Patient’s Myeloma Cell Proliferation Before Treatment

The percentages of bone marrow myeloma cells in the S phase in vivo increased by 2.65-fold (from 1.7% to 4.5%) during the month preceding anti-IL-6 treatment (Fig 1A). The peripheral blood contained 7,700 cells/mm³ with 12% myeloma cells and 42% neutrophils. The peripheral blood
myeloma cells were not in the S phase. Thirty-six days before and on the day of the beginning of the anti-IL-6 treatment, bone marrow mononuclear cells were harvested and cultured for 5 days. In these cultures, a significant spontaneous myeloma cell proliferation occurred (15% of myeloma cells in the S phase on day -36 and 8% on day 0). This proliferation was completely dependent on IL-6 in vitro, being inhibited by the anti-IL-6 MoAbs and reinduced by an excess of rIL-6 (Table 1).

Clinical Observations

No major side effects related to the 2-month treatment were noted. The body temperature returned to normal values within 6 days after the initiation of the treatment (Fig 1B). The patient was first treated with the B-E4 MoAb. After 6 days of treatment with B-E4, the patient's clinical status had not significantly improved and so the more potent B-E8 MoAb was injected, which produced a slight improvement of the performance status (from 10% to 20%). The first stoppage of treatment (from day 17 to day 23) was clearly associated with a worsening condition, especially recurrence of bone pain, in association with worsening of the biologic parameters, making it necessary to resort to the B-E8 anti-IL-6 injections again. The patient's clinical status slightly improved and stabilized at 30% from day 24 to day 56, worsened from day 56, and the treatment was stopped on day 60. This treatment arrest was followed by a fulminant progression of the disease with fever (Fig 1B), fatigue, recurrence of bone pain, and hypercalcemia. Throughout the treatment, the patient remained dependent on red blood cell transfusions in a similar way to what was observed before and after the treatment.

BIOLOGIC STUDIES

Serum IL-6 levels

Very low serum IL-6 levels (0.3 U/mL) were detected on day -36 and on day -3 before treatment (Fig 2A). Injection of the B-E4 MoAb resulted in a rapid increase of serum IL-6 levels which paralleled the increase of B-E4 MoAb concentration (Fig 2C) and reached 1,600 U/mL IL-6 on day 5 of treatment (Fig 2A). However, this IL-6 activity was detected only with high dilutions of these sera (>1:30; Fig 2B) and no IL-6 activity could be detected in these same sera by using our IL-6-dependent XG-1 human myeloma cell line (Fig 2B). Injection of B-E8 MoAb, which has a stronger affinity with IL-6 than the B-E4 MoAb, completely inhibited this IL-6 activity, which remained undetectable until day 60. On day 63, 3 days after the second stoppage of B-E8 injections, a high circulating IL-6-like bioactivity was again detected. This serum IL-6-like activity was completely suppressed by the reinjection of the B-E8 MoAb for 3 days (from day 64 to day 67) and rose...
to very high levels after the definitive arrest of anti–IL-6 injections in parallel with the disappearance of circulating anti–IL-6 MoAbs (Fig 2, A and C). It is interesting to note that this IL-6-like activity, detected by using the B9 hybridoma assay, was always completely inhibited by the B-E8 anti–IL-6 MoAb.

Serum Concentrations of Anti-IL-6 MoAbs and Immunization

The B-E4 MoAb was detected in the patient’s serum immediately after the first injection and it rose to a peak concentration of 49 μg/mL after the seventh injection (120 mg on day 6) (Fig 2C). From day 6 to day 15 the concentrations of circulating anti–IL-6 MoAbs decreased to around 5 μg/mL following the reduction of the injected doses and increased again to 25 μg/mL after a daily injection of 20 mg B-E4 MoAb for 3 days (Fig 2C). During the first 6-day interruption of the treatment (from day 17 to day 23), the MoAb concentrations decreased to a minimal value of 0.5 μg/mL on day 23. They stabilized between 5 and 10 μg/mL for 25 days with daily injections of 8 mg B-E8 and disappeared with a 3-day half-life at the end of the treatment. Immunization against the anti–IL-6 MoAbs was evidenced by the detection of human IgM antibodies to B-E4 on day 10 (results not shown) and of IgG at the same date (Fig 2D). The emergence of this immunization was inversely correlated with the rapid decrease of circulating anti–IL-6 MoAbs (Fig 2, C and D). During day 10 and day 14, the immunization was clearly directed against B-E4 only and not B-E8 or a control IgG2b MoAb. Immunization against B-E8 MoAb was detected on day 15 (Fig 2D). Interestingly, the immunization against the BE-4 and BE-8 MoAbs peaked on day 30 and progressively decreased to undetectable levels until day 50 (Fig 2D).

Inhibition of the Myeloma Cell Proliferation and Reduction of the Myeloma Ig and of Serum Calcium Levels

After injections of the anti–IL-6, we observed a rapid and complete reduction of the proliferation of bone marrow myeloma cells in vivo (Fig 1A). On day 10, there were no longer any myeloma cells in the S phase as opposed to 4.5% myeloma cells in the S phase before treatment. No myeloma cell proliferation was detected on day 22 and day 45. On day 60, 2% myeloma cells in the S phase were found in agreement with a worsening of the patient’s clinical status (Fig 1A). Bone marrow myeloma cells harvested on days 10, 22, 45, and 60 spontaneously proliferated in short-term cultures in vitro (Table 1) and this spontaneous proliferation was completely inhibited by the anti–IL-6 B-E8 MoAb, even on day 60. The IgG monoclonal protein concentration increased before and during the first 8 days of treatment to 9.8 g/dL, it then decreased and stabilized at a plateau level of 7.5 g/dL throughout the treatment. It increased rapidly to 11 g/dL following the definitive stoppage of treatment (Fig 1A). Before treatment, the serum calcium levels were rapidly increasing (from 3.10 mmol/L at day −12 to 3.65 mmol/L at day 0; Fig 1B). Injections of the anti–IL-6 MoAbs blocked this increase and progressively reduced these levels to 3.25 mmol/L at day 22 (Fig 1B). A rapid increase (3.9 mmol/L), followed by a new reduction of the calcium levels (3.4 mmol/L), were observed in association with the first interruption of the treatment and the resumption of the injections. After dexamethasone injections, the serum calcium levels decreased to near-normal values (3 mmol/L). The second interruption of the anti–IL-6 treatment on day 60 was followed by a huge and rapid increase of the calcium levels (4 mmol/L), which was blocked by the 3-day reinjection of the anti–IL-6 MoAbs. The absolute number of circulating myeloma cells did not decrease during treatment. These cells did not proliferate.

Fig 2. Serum levels of IL-6, of anti–IL-6 MoAbs, and immunization. (A) Serum IL-6 levels. (B) Measurement of IL-6 activity using the B9 murine hybridoma cells or the XG-1 human myeloma cell line in the serum harvested at day 7. (C) Concentration of circulating anti–IL-6 MoAbs in the patient’s serum. (D) Immunization against the anti–IL-6 MoAbs. Human antibodies to the murine anti–IL-6 B-E8 anti–IL-6 MoAb.
and their number oscillated from 200 to 600 cells/mm$^3$ either before or throughout the treatment.

**Reduction of the Numbers of Circulating Platelets and Neutrophils During Anti–IL-6 Treatment**

After the injection of anti–IL-6 MoAbs, a progressive reduction by one half of the platelet number was observed until day 22 (Fig 1C). From day 24 to day 29, the platelet number increased to initial levels, probably reflecting the arrest of the anti–IL-6 injections from day 17 to day 23 (Fig 1C), and it was again reduced by one half after the reinjection of anti–IL-6 MoAbs until day 41. The neutrophil number was also progressively reduced by one half within 40 days following the initiation of the treatment (Fig 1C). After day 40, the patient received three courses of dexamethasone and the neutrophil and platelet numbers increased again (Fig 1C). The hemoglobin concentration was low before treatment (8.3 g/dL) and did not improve throughout the treatment (range: 7 to 12 g/dL).

**Measurements of Serum Acute-Phase Proteins and Polyclonal Igs During Anti–IL-6 Treatment**

During the month before treatment, high serum CRP levels were found (70 mg/L, normal value <7 mg/L; Fig 1D). CRP levels increased to 90 mg/L on day 1 of treatment, rapidly decreased to undetectable levels within 10 days, remained undetectable throughout the treatment, and increased again at the end of the treatment (Fig 1D); a1-AT levels decreased by 40% during treatment and increased at the end of the treatment (Fig 1D). The serum albumin concentration was low before treatment (24.9 g/L) and remained low (range 22.8 g/L to 27.8 g/L) throughout the treatment. Circulating IgA rapidly decreased from 0.42 g/L before treatment to undetectable levels (<0.07 g/L) within 20 days, remained undetectable until day 52, and returned to initial values within the last 10 days of B-E8 MoAb treatment (Fig 1E). A parallel, but less pronounced, decrease was found for circulating IgM (Fig 1E). Immunofixation studies indicated that these IgA and IgM were not monoclonal but polyclonal. Serum levels of C3 and C4 complement components also decreased by 50% until day 50 and reincreased in parallel with dexamethasone injections (Fig 1F).

**DISCUSSION**

This clinical trial demonstrates the feasibility of blocking IL-6 activity for 2 months without undesirable effects. It provides important insights into the biologic effects of IL-6 in vivo and, in particular, it shows that IL-6 was the major tumoral growth factor in this patient with fulminating multiple myeloma in vivo, which is in full agreement with previous observations in vitro. What are the major conclusions that can be drawn from this clinical trial with anti–IL-6 MoAbs?

The Anti–IL-6 MoAbs Blocked Myeloma Cell Proliferation for 45 Days In Vivo

The blockage of the myeloma cell proliferation for 45 days during anti–IL-6 treatment indicates that IL-6 was the major myeloma cell growth factor during this period in vivo as it was in vitro. In agreement with our previous studies, the present study challenges a previous report claiming that IL-6 was not involved in patients with advanced disease. In vitro, we have now studied more than 30 patients with advanced or terminal disease, and in all cases the in vitro myeloma cell proliferation was found to be dependent on IL-6. The reductions of the serum myeloma protein concentrations and of the calcium levels are other indicators that the anti–IL-6 treatment resulted in a transient tumor cytostasis and reduction in IL-6–related toxicities. The reduction of the serum calcium levels during treatment is in harmony with recent data showing a critical role of IL-6 in the cytokine-mediated bone resorption in vitro.

The Injections of the Anti–IL-6 MoAbs Were Very Well Tolerated During the Two-Month Treatment and No New Symptom Unrelated to Disease Progression Was Observed

With the anti–IL-6 treatment, an improvement of the patient’s clinical status was observed in correlation with the improvement of some major biologic parameters reflecting disease severity (myeloma protein and serum calcium).

The Doses of Anti–IL-6 MoAbs That Were Injected Were in Sufficient Amounts to Completely Block the Production of C-Reactive Protein Throughout the Treatment

As nothing was known about the ability of the anti–IL-6 MoAbs to neutralize IL-6 in vivo or about eventual side effects of this treatment, the doses of anti–IL-6 MoAbs were chosen to reach serum MoAb concentrations similar to those known to inhibit the myeloma cell proliferation in vitro, and were adjusted according to the patient’s clinical status. This resulted in serum concentrations of 40 to 50 µg/mL B-E4 MoAb and of 8 to 10 µg/mL B-E8 MoAb. In vitro, the B-E8 MoAb is about fivefold more potent than the B-E4 MoAb for neutralizing IL-6, and such concentrations were able to completely inhibit the proliferation of fresh myeloma cells for 30 different MM patients studied.

The current data show the difficulty in estimating the efficiency of the anti–IL-6 MoAbs to neutralize IL-6 in vivo by using IL-6 bioassays. Indeed, by using the B9 hybridoma assay, a rapid and huge increase in the serum IL-6 levels was found in parallel with an increase in the B-E4 MoAb serum levels whereas no IL-6 activity could be detected with an IL-6–dependent human myeloma cell line. A likely explanation is a dissociation of circulating IL-6/BE-4 MoAb immune complexes by the IL-6 receptors of the murine B9 cells, which are 20-fold more sensitive to IL-6 than the XG-1 human myeloma cell line, which allowed the B9 cells to proliferate when diluted sera were used, unlike when undiluted sera were used. In agreement with this hypothesis, the injection of the B-E8 MoAb, which has a stronger affinity with IL-6 than the B-E4 MoAb, completely inhibi-
After Two Months

The current study shows that the best indicator of the in vivo efficiency of the two anti-IL-6 MoAbs in neutralizing IL-6 is the serum concentration of CRP. Recent studies have shown that the production of CRP by human hepatocytes in primary culture is controlled by IL-6 in vitro. Within 10 days after injections of the anti-IL-6 MoAbs, serum CRP rapidly decreased and became undetectable throughout the treatment (<1 mg/L). Interestingly, serum concentration of α1-AT, another acute-phase protein with a 5-day in vivo life span similar to CRP, was less affected by the anti-IL-6 treatment, consistent with the fact that the production of this protein by human hepatocytes is less dependent on IL-6 than the production of CRP in vitro. Thus, the present study shows that the production of CRP by hepatocytes is completely dependent on IL-6 in vivo and that serum CRP concentrations are easily measurable indicators of the IL-6 bioactivity in vivo. We have recently found that the serum CRP level is a strong prognostic factor in patients with MM, an abnormal serum CRP level being associated with poor survival (R.B., unpublished observations, May 1990).

How Can the Resumption of the Myeloma Cell Proliferation After Two Months of Treatment Be Explained?

Two hypotheses might clarify the resumption of the proliferation of the tumoral cells on day 60. One hypothesis is the emergence of a tumoral clone unresponsive to IL-6 and no longer inhibited by anti-IL-6 MoAbs, as is the case for most of the autonomously growing human myeloma cell lines in vitro. This hypothesis does not appear likely as the in vitro proliferation of the patient’s myeloma cells harvested on day 60 was still completely dependent on IL-6, i.e., it was inhibited by the anti-IL-6 MoAbs. Another hypothesis is that the anti-IL-6 MoAb was not, at the end of the treatment, in sufficient concentration in the bone marrow to prevent myeloma cells from binding IL-6. The rapid increase of the serum concentration of myeloma protein after the second stoppage of the anti-IL-6 treatment on day 60, whereas no increase was observed after the first stoppage on day 14, indicates that the concentrations of anti-IL-6 were just as minimal for blocking the tumor progression at the end of treatment. In keeping with this hypothesis is the huge increase of the serum IL-6-like activity at the end of treatment. This may be due to an overproduction of IL-6 by the tumoral environment. It may also be due to the emergence of human antibodies against the B-E4 or B-E8 idiotypic determinants, although no detectable immunization was found at the end of treatment. We are now actively investigating this point. Whatever its origin, this huge increase in IL-6-like activity production at the end of treatment may explain why the anti-IL-6 MoAb concentrations were not sufficient at this time to inhibit myeloma cell proliferation in the bone marrow, whereas they were sufficient to inhibit the production of CRP by hepatocytes. Indeed, the bone marrow is the major site of IL-6 production in MM patients in vivo and IL-6 must circulate from the bone marrow to the liver to trigger hepatocytes to release CRP. Thus, IL-6 may be more easily bound by anti-IL-6 MoAbs before reaching the hepatocytes as compared with the myeloma cells, which proliferate in close contact with the IL-6-producing tumoral environment.

Injection of the Anti-IL-6 MoAbs Resulted in a Decrease of Circulating Polyclonal IgGs

The complete suppression of the serum polyclonal IgA and the partial suppression of the serum polyclonal IgM during anti-IL-6 treatment are intriguing. Previous demonstrations that IL-6 is a central cytokine for the IgA production in vitro could explain these in vivo observations. The rapid increase of the polyclonal IgA and IgM during anti-IL-6 treatment on day 55 is also intriguing. This could be due to dexamethasone treatment because dexamethasone has been shown to increase the IL-6-induced Ig production in vitro.

The Numbers of Circulating Platelets and Neutrophils Were Decreased by One Half During Anti-IL-6 Treatment Without Hemorrhagic or Infectious Problems and Without Need for Transfusions During the Treatment

IL-6 stimulates the formation of megakaryopoietic colonies in vitro. More recently, the injection of rIL-6 to primates was shown to increase megakaryopoiesis in vivo. These data may explain why the variations in the number of circulating platelets almost exactly reflected, with a time delay, the variations in the serum concentrations of anti-IL-6 MoAbs during the first 40 days. After 45 days, the platelet number increased after dexamethasone treatment. The circulating neutrophil number also progressively decreased during the first 40 days, in agreement with the hematopoietic activity of IL-6 reported in vitro and in vivo. After day 45, the neutrophil number rapidly increased after dexamethasone treatment, probably owing to a release of marginating neutrophils. Hematopoiesis is controlled by a whole set of cytokines with overlapping functions and acting in synergy. This fact may explain why a suppression of the IL-6 activity in vivo did not result in a complete suppression of hematopoiesis in vivo.

In conclusion, the present study shows a blockage of the myeloma cell proliferation, and also a transient tumor cytostasis and a reduction in IL-6-related toxicities in this patient with terminal disease. IL-6 is also a growth factor for murine plasmacytomas in vitro and it has been recently demonstrated, in a similar way, that anti-IL-6 MoAbs can block plasmacytoma growth in mice in vivo. In addition, this study shows the feasibility of neutralizing IL-6 activity for 2 months without the occurrence of major side effects and it provides important insights into the biology of IL-6 in vivo. These last points are of importance for the further development of anti-IL-6 therapy not only in myeloma but also in other IL-6-related diseases: septic shocks, rheumatoid arthritis, proliferative mesangial glomerulonephritis, renal cell carcinomas, and Kaposi’s sarcoma.
REFERENCES


Murine anti-interleukin-6 monoclonal antibody therapy for a patient with plasma cell leukemia

B Klein, J Wijdenes, XG Zhang, M Jourdan, JM Boiron, J Brochier, J Liautard, M Merlin, C Clement and B Morel-Fournier