Role of Interleukin-6 in the Proliferation of Human Multiple Myeloma Cell Lines OCI-My 1 to 7 Established From Patients With Advanced Stage of the Disease

By J.K. Hitzler, H. Martinez-Valdez, D.B. Bergsagel, M.D. Minden, and H.A. Messner

Interleukin-6 (IL-6) has been shown to stimulate the proliferation of multiple myeloma cells purified to a high degree from human bone marrow. IL-6 production in multiple myeloma has been attributed to cells belonging to the myeloma clone, thus supporting a mechanism of autostimulation. In addition, it has been shown that IL-6 may be produced by auxiliary cell populations of the bone marrow that are not part of the myeloma clone. A definitive separation of both putative sources for IL-6 may be difficult to achieve in fresh patient samples. The purpose of this study was to investigate the IL-6 growth requirement and production by pure myeloma cell populations using seven human myeloma cell lines (OCI-My 1 to 7) that were established from patients with advanced disease. The proliferative response of each line to recombinant IL-6 was measured in a clonogenic assay providing human plasma and methylcellulose as a viscous support and by "H-thymidine uptake in liquid suspension culture. We observed marked heterogeneity, ranging from IL-6-depen-

Dent colony formation by OCI-My 4, to IL-6-independent growth. All lines expressed mRNA for the IL-6 receptor. Expression of IL-6 mRNA was analyzed after amplification by polymerase chain reaction and was present in five of seven lines. IL-6 protein was detected by enzyme-linked immunosorbent assay (ELISA) in the culture supernatants of two lines (OCI-My 3 and 2). Its functional activity was confirmed in a bioassay using the IL-6-dependent murine hybridoma line B 13.29. This activity was neutralized by anti-IL-6 antibody. Two lines did not express mRNA for IL-6. The remaining three lines expressed mRNA for IL-6, but did not secrete IL-6 protein. Immunoprecipitation experiments with lysates of one of these three lines did not detect the presence of IL-6 protein. These results suggest that autocrine stimulation by IL-6 may occur in some cell lines derived from patients with multiple myeloma. However, it does not represent a universal mechanism in myeloma cell growth.

INTERLEUKIN-6 (IL-6), also called B-cell differentiation factor (BCDF), B-cell stimulatory factor 2 (BSF-2), hybridoma/plasmocytoma growth factor, interferon (IFN-β), β2, interleukin-6 receptor on myeloma cells. The proliferation of IL-6-responsive myeloma cell populations was inhibited by anti-IL-6 antibodies. In addition, IL-6 activity was detected in the supernatants of myeloma cell cultures, suggesting an autocrine production of IL-6.

Other investigators confirmed the proliferative response of human myeloma cells to IL-6. However, in contrast, they identified adherent bone marrow cells as a primary source of IL-6. This observation is consistent with a paracrine production of IL-6 by normal cells.

We studied seven human multiple myeloma cell lines as a model for a self-renewing myeloma cell population. The lines were established in this laboratory from patients with advanced-stage multiple myeloma. The proliferative response of myeloma cells to exogenous IL-6 and production of IL-6 protein was examined. These parameters were correlated with cell kinetic data and clinical measurements.

MATERIALS AND METHODS

Cell culture. Human multiple myeloma cell lines (OCI-My 1 to 7) were established from bone marrow or peripheral blood samples of patients with advanced multiple myeloma. Six of these cell lines were generated from myeloma cell colonies grown in methylcellulose (Dow Chemical, Montreal, Quebec) in the presence of heparinized normal human plasma and medium conditioned by phytohemagglutinin (Wellcome Diagnostics, Dartford, UK)-stimulated leukocytes (PHA-LCM). One line was directly initiated in liquid suspension culture. The methods were previously described in detail. The lines were propagated in Iscove's medium (IDMEM; GIBCO, Grand Island, NY) supplemented with 20% human plasma and 50 μg/ml L-2-mercaptoethanol (2-ME; Sigma, St Louis, MO). All lines have retained their clonogenicity in methylcellulose. Growth curves were obtained from liquid suspension cultures in IDMEM, supplemented with 10% fetal bovine serum (Flow Laboratories, Rockville, MD) and 50 μg/ml L-2-ME. Cells were cultured at initial cell densities of 10⁵, 10⁶, and 10⁷ cells/ml, and cell numbers counted daily over a period...

From the Ontario Cancer Institute, Toronto, Ontario, Canada.

Supported by the National Cancer Institute of Canada, Medical Research Council of Canada, and Deutsche Forschungsgemeinschaft (DFG).

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of 4 days (electronic particle counter, built at the Ontario Cancer Institute, Toronto, Ontario).

**Colonies assay.** Myeloma cells were plated in 96-well microtiter plates (NuncLab, Nunc, Denmark) containing IMDM, 0.8% methylcellulose, 30% heparinized normal human plasma, and 50 μmol/L 2-ME. The final volume per well was 0.1 mL. When indicated, growth factors were added at the time of plating. Colonies were counted after 14 days of incubation at 37°C and 5% CO₂ in a humidified atmosphere.

**Preparation media conditioned by myeloma cells.** Based on previous experiments, cells were cultured at a cell density of 1 × 10⁷/mL in IMDM, 10% heparinized human plasma, and 50 μmol/L 2-ME for 72 hours under the conditions described above. Cells were removed by centrifugation and supernatants filtered (0.22 μm, Millipore-GV, Millipore, Bedford, MA) before storage at −20°C.

The same batch of human plasma was used for all cultures.

**[3H]-thymidine incorporation assay.** Myeloma cells were cultured at a concentration of 5 × 10⁴ and 5 × 10⁵/mL in IMDM supplemented with 10% heparinized human plasma and 50 μmol/L 2-ME for 48 hours at 37°C in a humidified atmosphere containing 5% CO₂. Methyl-[3H]-thymidine (46 Ci/ml; Amersham, Arlington Heights, IL) was added at a final concentration of 1 μCi/well 16 hours before cell harvest. All cultures were performed in triplicates at a volume of 0.2 mL per microtiter well.

The effect of anti-IL-6 antibody on myeloma cell proliferation was examined by adding goat anti-human IL-6 antibody at concentrations of 1/2,000, 1/400, 1/200, 1/100, and 1/50 (vol/vol) to the cultures at the time of their initiation (a generous gift from Dr Gordon Wong, Genetics Institute, Cambridge MA). Goat serum at identical concentrations was used as a control.

**IL-6 determination.** Two assays were used to quantitate IL-6 in myeloma cell-conditioned media. The supernatants were added to the IL-6-dependent murine hybridoma line B 13.29.12,25 Proliferation was evaluated by [3H]-TdR uptake as described above. One unit was defined as half-maximal stimulatory effect. The sensitivity of this assay was determined by a dose-response curve using 10⁻³ to 10⁻⁹ vol/vol dilutions of a human recombinant IL-6 stock solution (21 μg/mL or 7 × 10⁶ U/mg [CESS]; Genetics Institute, Cambridge MA; see Materials and Methods). The dose-response curve (log IL-6 dilution [vol/vol] v proliferative response measured in counts per minute) had a sigmoid shape, with the half-maximal effect (ED₅₀) measured at a 10⁻³ vol/vol dilution, corresponding to 210 pg/mL IL-6. Plateau values were reached at a 10⁻⁴ vol/vol dilution (2,100 pg/mL IL-6). The lower limit of sensitivity was determined at a 10⁻⁵ vol/vol dilution of IL-6 (equivalent to a concentration of 2.1 pg/mL), and the assay was used in the range thus specified.

The materials were also tested by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit for IL-6 (Quantikine: R&D Systems, Minneapolis, MN). This assay detects IL-6 in concentrations ranging from 10 to 2,000 pg/mL; in our experiments, the half-maximal value for optical density (ED₅₀) was measured at an IL-6 concentration of 880 pg/mL. In addition, a kit of this assay was determined by a dose-response curve using 10⁻¹ to 10⁻⁵ dilution (2,100 pg/mL IL-6). The lower limit of sensitivity was reached at a vol/vol dilution of IL-6 concentration of 0.015 ρmol/L, and the assay was used in the range thus specified.

**Immunoprecipitation.** The quantity of 10⁷ exponentially growing cells was labeled with 0.2 mcI/mL [³⁵S]-methionin (> 800 Ci/mmO, Amersham) for 1 hour at 37°C in 500 μL methionin-free minimal essential medium (MEM). Cells were spun, resuspended in 300 μL cold lysis buffer (1% Nonidet P-40, 150 mmol/L NaCl, 20 mmol/L Tris [pH 8.0], and 0.5 mmol/L phenyl methyl sulfonyl fluoride [PMSF, Sigma]) and kept on ice for 30 minutes. Lysates were centrifuged and supernatants precleared with 5 μg of a nonspecific murine monoclonal IgG2 antibody (Sigma). This procedure was performed overnight at 4°C, followed by incubation with 300 μL 10% formalin-fixed Staphylococcus aureus cells (Immunoprecipitin; BRL, Gaithersburg, MD) for 15 minutes on ice. A lysate volume equivalent to 10⁷ TCA-insoluble cpm was reacted with a polyclonal goat anti-human IL-6 antibody for 2 hours at 4°C. Goat serum was used as a control.

Immunocomplexes were collected by incubating samples with 50 μL S aureus cells for 15 minutes on ice and subsequent centrifugation. Sediments were washed twice in NET2 gel buffer (150 mmol/L NaCl, 5 mmol/L EDTA [pH 8.0], 50 mmol/L Tris [pH 7.4], 0.05% Nonidet F-40, 0.02% sodium azide, 0.25% gelatin), eluted into 30
μL sample buffer (2% SDS, 0.1% bromophenol blue, 10% glycerol, 25 mM/L Tris [pH 6.8], 0.1 mol/L dithiothreitol) by heating for 10 minutes at 70°C and loaded onto a 14% SDS-polyacrylamide gel. Electrophoresis was performed for 2 hours at 55 mA in Tris-glycine buffer (0.3% Tris, 1.4% glycine, 0.1% SDS). Gels were stained for 30 minutes in Coomassie blue stain (0.25% Coomassie brilliant blue, 50% methanol, 10% acetic acid), destained overnight in 10% acetic acid, dried, and exposed to Kodak X-AR5 for 1 to 3 days at −70°C. The position of human recombinant IL-6 electrophoresed on the same gel was visualized by Coomassie blue staining and marked accordingly on the final x-ray film.

Sources of materials. Human recombinant IL-6 (produced in E coli; stock solution 21 ng/mL, 7 × 10⁶ U/mg [CESS]) and a polyclonal goat anti-human IL-6 antibody were a generous gift from Drs S. Clark and G. Wong, Genetics Institute, Cambridge, MA. The same batch of antibody was used for neutralization, immunoprecipitation, and [³H]-Tdr uptake experiments. From the same source were cDNA probes for GM-CSF³⁷ and IL-1β.³⁶ The cDNA used as IL-6 receptor probe³⁶ was kindly provided by Dr T. Kishimoto, Osaka, Japan. Oligonucleotides used as primer molecules and probes were purchased from the Oligonucleotide Synthesis Laboratory at Queen’s University, Kingston, Ontario.

RESULTS

Secretion of IL-6 protein. Supernatants conditioned by the seven human myeloma cell lines were tested for IL-6 activity using two different assays. The supernatants of OCI-My 2 and 3 promoted growth of the IL-6-dependent murine hybridoma line B 13.29 (Fig 1). By ELISA, the concentrations were determined as 48 and 156 pg/mL, respectively. The activities elaborated by both lines could be neutralized by anti-human IL-6 antibody preparations. A representative experiment for OCI-My 3 is shown in Fig 2. Supernatants of all other lines did not yield any detectable IL-6 activity by bioassay or ELISA. Culture supernatants from all cell lines were also tested for IL-1β as detected by ELISA. None of the supernatants showed activity (data not shown). The effect of anti-IL-6 antibody on IL-6–secreting and nonsecreting myeloma cell lines was

![Fig 1. \[^{3}H\]-Tdr incorporation of B 13.29 cells in response to medium conditioned by cell lines OCI-My 1 to 7. B 13.29 E cells, at a concentration of 5 × 10⁶/mL, were cultured in the presence of (■) 5%, (□) 10%, and (△) 20% medium conditioned (CM) by the human myeloma lines OCI-My 1 to 7. Mock conditioned medium (0) and human recombinant IL-6 at 10⁻², 10⁻¹, and 10⁻³ vol/vol dilutions (hrIL-6) were used as controls. Values represent means of triplicate measurements. Three preparations of conditioned medium were tested for each cell line. One representative experiment is shown.](http://www.bloodjournal.org)

![Fig 2. Neutralization of growth-promoting activity in OCI-My 3 conditioned medium by anti-IL-6. [³H]-Tdr uptake of the IL-6–dependent murine hybridoma cell line B 13.29 at a concentration of 5 × 10⁶/mL was measured in the presence of 10% medium conditioned by OCI-My 3. Values obtained for supernatants treated with a polyclonal goat anti-human IL-6 antibody (■) or goat serum in identical dilutions as a control (○) are compared with untreated OCI-My 3 conditioned medium (9,796 cpm = 100%). Incorporation in the absence of any conditioned medium was measured at 2,213 cpm.](http://www.bloodjournal.org)

![Fig 3. IL-6 mRNA expression in OCI-My 1 to 7. After reverse transcription of 1 μg total cellular RNA, cDNA from all cell lines was amplified by polymerase chain reaction and probed with oligonucleotides specific for IL-6. Cell lines OCI-My 1, 2, 3, 6, and 7 express mRNA for IL-6, whereas OCI-My 4 and 5 are negative. RNAs from the bladder carcinoma line 5637 and the lymphoma cell line OCI-Ly 12, which secretes IL-6 in an autocrine fashion, were used as positive controls.](http://www.bloodjournal.org)
tested in three lines. OCI-My 3 was selected as IL-6-secreting line, OCI-My 1 represents an IL-6 mRNA-positive nonsecreting line, and OCI-My 5 was used as an IL-6 mRNA-negative control cell line. Proliferation was measured by $[^3H]$-TdR uptake in the presence of anti–IL-6 antibody at concentrations ranging from 1/200 to 1/50 (vol/vol) or goat serum as a control. Proliferation was not altered in the presence of anti–IL-6 antibodies (data not shown).

**IL-6 mRNA expression.** Total cellular RNA of all lines was examined for the presence of IL-6 mRNA. The sensitivity was increased by including an amplification step using the polymerase chain reaction technique.

IL-6 mRNA was observed in five of the seven lines, but only two cell lines (OCI-My 2 and 3) secreted biologically active protein. OCI-My 4 and 5 did not express mRNA for IL-6 (Fig 3). Message for IL-1β and granulocyte-monocyte colony-stimulating factor (GM-CSF) was not expressed as determined by Northern analysis of all cell lines (data not shown).

**Immunoprecipitation of intracellular IL-6.** Based on these studies, the question was asked whether cytoplasmic IL-6 could be identified in IL-6 mRNA-positive nonsecreting lines.

Cell lysates were prepared from three different myeloma cell lines. OCI-My 1 was chosen as IL-6 mRNA-positive, IL-6-nonsecreting myeloma cell line. The IL-6 mRNA-negative, IL-6-nonsecreting cell line OCI-My 5 and the IL-6-secreting line OCI-My 3 were included as negative and positive controls. The lysates were tested in concentrations ranging from a 1/500 to a 1/2 (vol/vol) dilution for IL-6 activity using the B13.29 bioassay. IL-6 activity could not be detected in any lysate (data not shown).

The same question was addressed in a second series of experiments. Cells of the same three cell lines were pulse-labeled with $[^35S]$-methionine followed by immunoprecipitation of the lysates using an antibody preparation against IL-6 (Fig 4). Lanes denoted as “b” contained protein precipitated with a polyclonal goat anti-human IL-6 antibody, and lanes identified as “a” showed control precipitates prepared with nonspecific goat serum. Separation of human recombinant IL-6 protein in a separate lane and Coomassie blue staining indicated the position of IL-6 protein (Fig 4).

A band specific for IL-6 was present in the IL-6-secreting cell line OCI-My 3 (lane b). The IL-6 mRNA-negative cell line OCI-My 5 did not show any band in a similar position. In cell line OCI-My 1, both the specific antibody (b) and the
non specific serum (a) precipitated a faint band that was located distinctly below the position of IL-6 protein. These two observations are consistent with a non specific band.

**IL-6 receptor mRNA expression.** To gain further information about the interaction between the available myeloma cell lines and IL-6, we examined the presence of mRNA for the IL-6 receptor and the proliferative response to IL-6 by direct addition of IL-6 to the culture. Northern blot analysis of all seven cell lines showed the presence of the specific 5-kb IL-6 receptor mRNA at various levels of expression (Fig 5). This was confirmed by amplification of a cDNA fragment corresponding to a 600-bp nucleotide sequence of the human IL-6 receptor mRNA by polymerase chain reaction (data not shown).

**Proliferative response to IL-6 in cell lines OCI-My 1 to 7.** Cell growth of the myeloma cell lines in response to exogenous IL-6 was examined by colony formation and [H]-TdR uptake. Cells of each line were plated in semi-solid medium containing human recombinant IL-6 at a concentration ranging from 0.0002 to 200 U/mL as determined by the bioassay using the IL-6-sensitive murine hydroma cell line B 13.29. Three types of response were observed.

Colony formation by OCI-My 4 was completely dependent on addition of IL-6 when cells were plated at low concentrations (Fig 6A depicts the dose-response curve for 1 x 10^4 cells/mL). The colony frequency is increased nearly eight times in the presence of optimal concentrations of IL-6. Cells grown at higher concentrations (5 x 10^4/mL) gave rise to a small number of background colonies without IL-6 (Fig 6B). However, the addition of IL-6 resulted in a dose-dependent increase in colony formation similar to that observed at lower cell number. Used in a bioassay for IL-6, OCI-My 4 is less sensitive than the murine hybridoma B 13.29.

The cell lines OCI-My 7 and OCI-My 6 formed some colonies spontaneously in the absence of IL-6, even if plated at low cell concentration (1 x 10^4/mL). Addition of exogenous IL-6 resulted in a twofold to threefold increase in the number of colonies. Furthermore, the size of colonies formed by OCI-My 7 cells increased proportional to the IL-6 concentration added (Fig 7). The frequency and size of colonies formed by OCI-My 3, OCI-My 1, and OCI-My 5 was not enhanced by exogenous IL-6.

The proliferative activity of all lines in response to IL-6 was measured by [H]-TdR incorporation. The lines OCI-My 4, 7, 6, and 2 showed a 1.9- to 2.8-fold increase in [H]-TdR incorporation. The results obtained for OCI-My 4, 7, and 6 were consistent with increases observed for colony formation. OCI-My 2 was the only line demonstrating increased growth in liquid suspension culture without enhanced colony formation.

Cell growth in the absence of exogenous IL-6 was monitored in liquid suspension culture, supplemented with fetal bovine serum at cell densities between 10^5 and 10^6 cells/mL. Three cell lines were selected for specific studies: IL-6 protein-secreting line OCI-My 2, the IL-6 mRNA-positive nonsecreting line OCI-My 1, and the IL-6 mRNA-negative line OCI-My 5. None of the lines showed a lag phase when evaluated to determine the growth kinetics, even if cells were cultured at low cell density (data not shown).

**Correlation of response to IL-6 and doubling time.** We examined the question whether or not the proliferative response to IL-6 was correlated with other characteristics of the cells such as doubling time. The response to IL-6 of each cell line as measured by colony formation and [H]-TdR uptake was expressed as stimulation index. This was defined as test result with optimal concentration of IL-6 over test result without IL-6. The index was used to rank all lines for each test separately in decreasing order of stimulation. A combined rank (mean of both ranks for each line) was computed and compared with doubling time. Cell lines that showed a poor response to IL-6 tended to have a shorter doubling time (Table 1).

**DISCUSSION**

Seven human myeloma cell lines were used as a model system to evaluate the role of IL-6 in this disorder. Because
Fig 6. Colony formation of OCI-My 4 cells in response to IL-6. (A) At a cell concentration of $1 \times 10^3$ cells/mL, IL-6 stimulated colony formation in a dose-dependent manner. PHA-LCM (10% vol/vol) had a similar effect. Colony formation was not observed in the absence of IL-6 (control). (B) At a higher cell concentration ($5 \times 10^3$ cells/mL), a small number of background colonies was observed in the absence of IL-6.

Fig 7. Myeloma colonies formed by OCI-My 7 cells in the absence and presence of IL-6. Cells were plated at $5 \times 10^3$ cells/mL and cultured for 14 days (A) without IL-6 and (B) with $10^{-3}$ vol/vol IL-6. Colony number and size of OCI-My 7 cells are increased in cultures containing IL-6.
the lines were derived from patients with aggressive advanced disease, conclusions cannot be generalized.

Asaoi et al. reported a decrease in stimulation indices for IL-6 in freshly obtained myeloma cells from patients with advanced disease. In contrast, Zhang et al. using labeling index as a parameter of disease progression, showed a high proliferative response to IL-6 in this category. Our data demonstrated marked heterogeneity in the proliferative response to IL-6 measured by colony formation and/or \( ^{3}H \)-TdR uptake. The response ranged from growth being IL-6-dependent to IL-6-independent proliferation. The proliferative response did not correlate with the expression of IL-6 receptor mRNA in these lines. The latter is consistent with observations by Kawano et al. IL-6 responses were seen in cell lines with comparatively longer doubling times. It has been suggested that as part of disease progression, the growth responsiveness of cells changes. The seven lines described in this report arose toward the end of the life of each of the patients; there was no difference in the time from establishment of a cell line to time of death. However, there are differences in the cell behavior depending on time from diagnosis to time at which a cell line could be established. Patients whose cells were responsive to IL-6 had a longer time to progression than patients whose cells responded poorly to IL-6. Although we are dealing with small numbers, this suggests that the mechanism of transformation differs in the two groups.

As previously reported, IL-6 may be produced by human myeloma cells, thus supporting a self-stimulatory mechanism. Our study demonstrated the constitutive expression and secretion of IL-6 protein in two of the seven lines. Production by OCI-My 3 was sufficient to promote colony formation by IL-6–dependent line OCI-My 4. However, anti–IL-6 antibodies did not influence the proliferative pattern of OCI-My 3 cells. The addition of anti–IL-6 antibodies also did not influence cell lines that contained message for IL-6, but did not produce the protein. This observation suggests that autocrine growth stimulation by IL-6 may occasionally, but not always, occur in human myeloma. This view is supported by some of the other results of our studies. For instance, the production of IL-6 did not correlate with the proliferative response to IL-6. The two lines that did not express mRNA for IL-6 occupied opposite ends of the spectrum that ranked lines according to their IL-6 stimulation index. Growth curves for IL-6–secreting and -nonsecreting lines did not show a lag phase, even if cells were plated at low cell concentrations. The absence of such a lag phase provides further evidence for the view that the autocrine production of IL-6 is of less importance than initially assumed.

The expression of IL-6 mRNA in three lines that do not secrete detectable amounts of IL-6 protein requires further exploration. It is conceivable that the available bioassays are not sensitive enough to detect minute quantities of biologically active material. Alternatively, the message for IL-6 may not be functional. This would require a detailed structural analysis. Findings by Dunbar et al. suggest a third possibility. In this study, IL-3–dependent murine 32D cells were rendered growth factor-independent after transfer of a modified IL-3 gene, resulting in production and intracellular retention of the modified IL-3 protein. To address this question, cell lysates of IL-6–secreting myeloma cells were compared with the IL-6 activity in lysates of nonsecreting IL-6 mRNA-positive, as well as IL-6 mRNA-negative, myeloma cells. IL-6 activity was not detected. One possible explanation of this observation is a rapid degradation of IL-6 protein by simultaneously released protease activity. To investigate this further, an immunoprecipitation method was used. A band specific for IL-6 was observed for the IL-6–secreting myeloma line OCI-My 3 and was absent in the IL-6 mRNA-negative nonsecreting line OCI-My 5. The IL-6 mRNA-positive, IL-6–nonsecreting line OCI-My 1 did not yield any immunoprecipitable cytoplasmic IL-6 protein. Within the constraints of the methods, the observations do not support the presence of an alternative pathway restricted to the private domain. This could be further explored in experiments testing the effect of IL-6 antisense oligonucleotides on myeloma cell proliferation.

Alterations such as differences in IL-6 sensitivity of myeloma cell proliferation or paracrine production of IL-6 and other factors seem to play a more significant role. This is suggested by our studies, as well as by observations described by Klein et al. They noticed that, during their early generations, myeloma cell lines may require the presence of GM-CSF in addition to IL-6. This response to a second factor was lost after several cell divisions. Furthermore, cell lines from some patients with advanced disease did not require IL-6.

Further detailed longitudinal studies are necessary to determine if the various responses reflect a specific state in disease development or whether individual patients may follow individual patterns that do not change as the disease progresses. The latter would make a further identification of actual regulatory controls and their discrimination from epiphenomena more complicated.

### Table 1. IL-6 Responsiveness and IL-6 Production of Myeloma Cell Lines OCI-My 1 to 7

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*Doubling time was shortened to 28 hours when PHA-LCM was added to the cultures.
†Secreted into the culture supernatant.
REFERENCES

32. Takahashi T, Lim B, Wandl U, Jamal N, Nguyen A, Benchimol S, Bergsagel DE, Messner HA: Human myeloma cell lines generated from myeloma colonies of bone marrow or peripheral blood samples. (manuscript in preparation)
Role of interleukin-6 in the proliferation of human multiple myeloma cell lines OCI-My 1 to 7 established from patients with advanced stage of the disease

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