Interleukin-6 Is a Potent Myeloma-Cell Growth Factor in Patients With Aggressive Multiple Myeloma

By X.G. Zhang, B. Klein, and R. Bataille

It has recently been demonstrated that interleukin-6 (IL-6) is a potent myeloma-cell growth factor in the majority of patients with multiple myeloma (MM). Using an antibrivomodeoxyuridine monoclonal antibody (MoAb) to specifically count myeloma cells in the S-phase (i.e., labeling index, LI), we demonstrate that the IL-6 responsiveness of myeloma cells in vitro is directly correlated with their LI in vivo. Myeloma cells from all 13 patients with high LIs in vivo (≥1%) responded in vitro to IL-6, the strongest response occurring in cells from five patients with plasma-cell leukemia. In contrast, the cells of only two of eight patients with lower myeloma-cell LIs in vivo (<1%) responded to IL-6 in vitro. After seven days of culturing with 1,000 U/mL recombinant IL-6 (rIL-6), the median LI value in the first group of patients (in vivo LI ≥1%) was 11%, ie, 11 times higher (P < .01) than the median LI value (1%) in the second group of patients (in vivo LI <1%). Thus, the in vitro IL-6 responsiveness of myeloma cells is directly related to their in vivo proliferative status, and hence to the severity of the disease.

INTERLEUKIN-6 (IL-6) is a pleiotropic cytokine with a wide range of biological activities in T cells, B cells, hepatocytes, fibroblasts, hematopoietic cells, and neural cells. It is produced by many cell types including monocytes, fibroblasts, T cells, B cells, endothelial cells, and various tumor cells. Most interestingly, IL-6 is a potent growth factor for murine plasmacytomas and B-cell hybridomas, and we and others have shown that it is a potent growth factor for human myeloma cells. Myeloma cells proliferate poorly in vivo in patients with multiple myeloma (MM). Several studies have shown that the in vivo myeloma-cell labeling index (LI: percentage of myeloma cells in the S-phase) is one of the best prognostic indicators, ie, a high myeloma-cell LI indicates poor prognosis. In the present study, we have demonstrated that the IL-6 responsiveness of myeloma cells in vitro is directly correlated with their proliferative status in vivo. IL-6 was found to be a potent myeloma-cell growth factor in patients with proliferating myeloma cells in vivo (LI >1%). In contrast, IL-6 had little effect on myeloma-cell proliferation in patients with LIs below 1% in vivo.

MATERIALS AND METHODS

Patients. Response to IL-6 was studied in 21 patients with malignant plasma-cell dyscrasias, including 16 patients with MM and five with a plasma-cell leukemia (PCL). The diagnostic criteria were those of the Southwest Oncology Group of the USA. Patients with PCL had more than 20% malignant plasma cells in their peripheral blood. All patients were studied during an active phase of disease, either at diagnosis (nine cases) or during disease progression (12 cases). At the time of the study, 16 of 21 patients had stage III MM, four had stage II MM, and one had stage I MM.

Proliferation assay of myeloma cells. Bone-marrow and peripheral-blood (PCL) samples were harvested by iliac or venous punctures after obtaining the patients’ informed consent, and mononuclear cells were isolated by Ficoll-hypaque gradient centrifugation. Bone marrow cells were cultured for seven days in Iscove culture medium supplemented with 5 x 10⁻¹ mol/L 2-mercaptoethanol and 5% fetal calf serum (FCS) in the absence or presence of various concentrations (100, 500, 1,000 U/mL) of rIL-6 provided by L. Aarden (Amsterdam, The Netherlands). At the initiation of cultures and on culture day 7, the percentages of myeloma cells were determined by intracytoplasmic immunofluorescence using anti-kappa or anti-lambda light chain antibodies bound to fluorescein (Kallestadt, Austin, TX). The percentages of myeloma cells in the S-phase were determined using an antibrivomodeoxyuridine monoclonal antibody (MoAb) (Bu-1, a generous gift from N. Gonchoroff, Mayo Clinic, Rochester, MI) and a rhodamine-labeled goat anti-mouse immunoglobulin (Cappel Laboratories, Malvern, PA) in a double fluorescence technique described elsewhere.

RESULTS

As shown in Figure 1, 13 patients had significant levels of proliferating myeloma cells in vivo (LI ≥1%). Samples from all these patients showed a spontaneous increase in myeloma-cell proliferation after seven days of culture (Fig 1). The median LI value on culture day 7 was 6% (range 3% to 10%), which was three times higher (P < .01) than the median LI value on day 0 (2%, range 1% to 5%). In previous experiments, we have shown that this spontaneous increase in myeloma-cell proliferation is due to endogenous IL-6 produced in these cultures: it was abrogated by anti–IL-6 antibodies and reinduced by rIL-6. In this group of patients (in vivo LI ≥1%), exogenous rIL-6 (1,000 U/mL) further increased myeloma-cell proliferation (P ≤ .01) with a median LI value of 11% (range 2.5% to 25%). As shown in Fig 1, exogenous rIL-6 induced a high myeloma-cell proliferation in all five patients with PCL: the median LI value of myeloma cells cultured seven days with 1,000 U/mL rIL-6 was 11%, with notably 25% of the myeloma cells in the S-phase in one patient after IL-6 stimulation.

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Eight patients had very few or no proliferating myeloma cells in vivo (ie, LI < 1%, Fig 1). After seven days of culture with IL-6 (1,000 U/mL), the myeloma-cell LI remained very low (LI ≤ 1%) in cells from six of eight patients and the median LI value was 1% (range 0% to 5%) (Fig 1). It was 11 times lower (P < .01) than the median myeloma-cell LI value (11%) found in the first group of patients under the same culture conditions (see above).

**DISCUSSION**

In patients with MM, myeloma cells do not proliferate or proliferate very little in vivo. Previous studies have shown that the percentage of myeloma cells in the S-phase in vivo (LI) is one of the best prognostic indicators, ie, a high myeloma-cell LI indicates poor prognosis, independently on the initial tumor cell mass. Our recent results demonstrate that IL-6 is a potent growth factor essentially in patients with high in vivo myeloma-cell LIs (LI ≥ 1%), the strongest response occurring in patients with PCL. In contrast, IL-6 induced little or no myeloma-cell proliferation in patients with low myeloma-cell LIs in vivo. Asaoku et al have recently reported that myeloma-cell response to IL-6 was optimal in patients with low cell mass MM (stage I MM), and decreased in patients with advanced disease (stage III MM). These authors concluded that during progression of disease a majority of cells decrease their dependence on growth factor and subsequently display a self-controlled continued growth. In the present report, by studying mainly stage III MM and PCL, we come to the different conclusion that myeloma-cell response to IL-6 is optimal in patients with severe disease, independently of their tumor mass. As anti–IL-6 therapeutics (anti–IL-6 antibodies, anti–IL-6-receptor antibodies, IL-6 linked to a toxin) could be used for treating MM, it is essential to understand these differences. The first source of discrepancy is the use by Asaoku et al of the Durie-Salmon staging (ie, myeloma cell mass) as the only indicator of disease severity. This staging is not presently the best indicator of disease progression compared with the in vivo myeloma-cell LI used in the present study. Second, Asaoku et al took, as an indicator of IL-6 responsiveness, the increase in myeloma-cell proliferation on day 2 of culturing in the presence of exogenous rIL-6, compared with the spontaneous proliferation of these myeloma cells on day 2 of culturing without exogenous IL-6. Since they showed in a previous paper, concurrently with us, that the spontaneous proliferation of myeloma cells in culture is mediated by endogenous IL-6 produced in the cultures, they actually measured the stimulation index of exogenous rIL-6 v endogenous IL-6, and not IL-6 responsiveness. Third, another source of discrepancy is the use of tritiated thymidine incorporation in their proliferation assay, instead of a direct determination of myeloma cells in the S-phase, as in our study. IL-6 is a growth factor for many cell lines and since it is very difficult to purify myeloma cells, the use of tritiated thymidine incorporation does not allow a specific evaluation of myeloma-cell proliferation.

Very interestingly, our data indicate that the absence of myeloma-cell proliferation in vivo in some patients with MM is associated with a lack of response by these myeloma cells to IL-6. This cannot simply be explained by a lack of IL-6 receptors, since myeloma cells have been found to express these receptors in patients not responsive to IL-6. It is crucial to determine the mechanisms underlying the IL-6 responsiveness/unresponsiveness of myeloma cells, since this phenomenon is directly correlated with in vivo myeloma-cell proliferation and disease severity. In previous studies, we have found significant amounts of IL-6 in the serum of patients with fulminating disease progression, especially those with PCL (R. Bataille, unpublished results, 1989). Furthermore, we have found that IL-6 production by bone marrow cells in patients with aggressive MM is greater than in patients with inactive MM. The present results demonstrating that IL-6 is a potent myeloma-cell growth factor in patients with in vivo proliferative MM, especially in those with PCL, suggest that anti–IL-6 antibodies, anti–IL-6-receptor antibodies, or IL-6 linked to a toxin could be used to induce remission in these patients.


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