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 antibromodeoxyuridine monoclonal antibody (MoAb) to specifically count myeloma cells in the S-phase (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 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.

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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.14-18 Our present results demonstrate that IL-6 is a poten 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). They concluded that during progression of disease a majority of cells decrease their dependence on growth factor and subsequently display a self-controlled continued growth.19 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.20 This staging is not presently the best indicator of disease progression compared with the in vivo myeloma-cell LI used in the present study.14-18 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 endogenous rIL-6, compared with the spontaneous proliferation of these myeloma cells on day 2 of culturing without endogenous IL-6. Since they showed in a previous paper,13 concurrently with us,14 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 by endogenous IL-6, and not IL-6 responsiveness. Third, another source of discrepancy is the use of tritiated thymidine incorporation in their proliferation assay,21 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 lines14-16 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-6.13 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.14 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, would 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.
REFERENCES


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