Decrease in BSF-2/IL-6 Response in Advanced Cases of Multiple Myeloma

By Hideki Asaoku, Michio Kawano, Koji Iwato, Osamu Tanabe, Hideo Tanaka, Toshio Hirano,
Tadamitsu Kishimoto, and Atsushi Kuramoto

Human myeloma cells freshly isolated from 40 patients with IgG multiple myeloma (MM) in stage I and 30 in stage III, were cultured for 48 hours with recombinant B cell stimulatory factor 2 (rBSF-2)/interleukin-8 (IL-6), which is considered a major growth factor for myeloma cells. Uptake of 3H-thymidine by these purified myeloma cells was measured, and BSF-2 response was evaluated by stimulation index and cpm induced by rBSF-2. Myeloma cells from cases in stage I responded to rBSF-2 better than those in stage III. Moreover rBSF-2 responders also showed better response to chemotherapy. Therefore, these results suggest that in vitro response of myeloma cells to BSF-2 correlates with disease progression and clinical response in patients of MM.

BSF-2 response was evaluated with the stimulation index, calculated as follows: Stimulation index – rBSF-2/spontaneous 3H-TdR uptake.

Treatment. MM patients in stage III were treated with alkylating agent [melphalan (MP) or cyclophosphamide (CP)] combined with prednisolone. The chemotherapy regimens (MP continuous, or MP intermittent administration and CP) were as follows: MP continuous, MP 2 mg orally every day and prednisolone 10 mg orally every other day; MP intermittent, MP 6 to 8 mg/m2 orally on days 1 through 4 and prednisolone 40 to 60 mg/m2 orally on days 1 through 4, 30 mg orally day 5, 15 mg orally day 6; CP, CP 150 mg/m2 orally on days 1 through 4 and prednisolone 40 to 60 mg orally on day 1 through 4, 30 mg on day 5 and 15 mg orally day 6. The courses in MP intermittent and CP therapy were given repeatedly in a cycle of between 3 and 4 weeks.

The response to the treatment was evaluated mainly according to the criteria proposed by the Committee of the Chronic Leukemia-Myeloma Task Force, National Cancer Institute. A partial response (PR) was recorded when >50% reduction of the serum M-protein or the urinary excretion of M-protein was observed. A minor response (MR) was defined as a decrease in serum or urinary M-protein within a range of 25% to 50%. Clinical response included not only partial response (PR) but minor response (MR) as well.

Statistical analysis. The statistical significance between mean values in the various groups was determined by Student’s t test.

RESULTS

Myeloma cells from patients with stage I MM were more sensitive to rBSF-2 than those from stage III. Ten myeloma cell samples from stage I multiple myeloma and 30 from stage III were purified, and their response to rBSF-2


From the Department of Internal Medicine, Research Institute for Nuclear Medicine and Biology, Hiroshima University; and the Division of Immunology, Institute for Molecular and Cellular Biology, Osaka University, Japan.

Submitted February 8, 1988; accepted March 15, 1988.


Address reprint requests to Michio Kawano, MD, Department of Internal Medicine, Research Institute for Nuclear Medicine and Biology, Hiroshima University, Kasumi 1-2-3, Minami-ku, Hiroshima 734, Japan.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1988 by Grune & Stratton, Inc.

0006-4971/88/7202-0045$3.00/0
was evaluated. Figure 1 shows representative profiles of response to rBSF-2 in stage I and III MM. In most responder cases, at least 20 U/mL rBSF-2 could induce maximal $^3$H-TdR uptake. Nonresponders to rBSF-2 showed not only low but also high spontaneous $^3$H-TdR uptakes. BSF-2 responses did not correlate with the level of spontaneous $^3$H-TdR uptake.

Thus, the stimulation index of myeloma cells from stage I MM patients had higher indexes (mean ± SE = 3.80 ± 0.95) than did those (1.85 ± 0.20) in stage III ($P < .01$), as shown in Fig 2.

These data show that myeloma cells in early stage I can respond to rBSF-2 better than those in advanced stage III; thus, BSF-2 responses of myeloma cells in vitro may correlate with progression of the myeloma disease.

Response to rBSF-2 correlated with clinical response. Thirty patients with stage III MM were treated with an alkylating agent (MP or CP) and prednisolone. Clinical response, which include PR and MR, was evaluated as described in the Materials and Methods section.

As shown in Fig 3, nonresponders to treatment, tended to have lower $\Delta$cpm, which indicates that $^3$H-TdR uptake by cells from nonresponders could not be augmented by rBSF-2 ($\Delta$cpm = 1,194.8 ± 589.5). On the other hand, cells from clinical responders similarly showed better response to rBSF-2 ($\Delta$cpm = 6,025.6 ± 1,926.8) ($P < .01$). This was also the case when rBSF-2 response was evaluated with the stimulation index: Stimulation index by rBSF-2 = 2.52 ± 0.34 (mean ± SE) and 1.34 ± 0.14 in treatment responders and nonresponders, respectively. Thus, treatment responders clearly had a significantly higher stimulation index ($P < .01$). These results suggest that in vitro response of myeloma cells to BSF-2 correlates positively with clinical response to treatment.

**DISCUSSION**

In a previous study, we demonstrated that only 50% of myeloma cells responded to BSF-2/IL-6, and the response was not related to the expression of BSF-2 receptor. In this study, we showed that myeloma cells in early stage I responded to rBSF-2 better than those of advanced stage III. Furthermore, response of these myeloma cells to BSF-2 tended to show changes corresponding with the disease progression. Thus, these results appear to provide important evidence in support of the general hypothesis that during progression of the disease a majority of tumor cells decreases the dependence on growth factor(s) responsible for promotion of their respective proliferation and/or differentiation and subsequently display a self-propelled continued growth. Recently, Wheeler and colleagues reported that introduction of the v-fms gene, which codes for a glycoprotein related to the receptor for colony-stimulating factor 1 (CSF-1), into simian virus-40 (SV40)-immortalized, CSF-1-dependent macrophages rendered them independent of CSF-1 for growth. These results suggest that v-fms product is unregulated, thus providing growth stimulatory signals in the absence of CSF-1. Hence, although no direct evidence
shows how BSF-2 nonresponding myeloma cells can proliferate spontaneously with autonomy, BSF-2 receptors, particularly in myeloma cells that show less or no response to BSF-2,

probably have either undergone an endogenously or self-induced transformation or merely a response to a misdirection by the signal transducing proteins that lie within the cells. Thus, we believe that purification and characterization of the BSF-2 receptor is a necessary step toward investigation of aberrantly activated receptors, which will lead to further understanding of the corresponding consequences. These studies are now in progress.

On the other hand, response of myeloma cells to rBSF-2 correlated well with therapeutic effects: Most BSF-2 responders similarly displayed clinically good therapeutic effects. Nonetheless, the exact mechanism involved in support of this relationship between the parallel response—the response to the rBSF-2 and the corresponding response to the therapeutic agents—is still not clear. Durie and co-workers previously indicated that the labeling index (LI%) with $^3$H-TdR is a valid independent prognosticator for survival duration$^{16}$ and further reported that the grain count over each $^3$H-TdR–labeled cell is related to the patient’s clinical responses. Moreover, spontaneous high $^3$H-TdR incorporation correlated highly with resistance to treatment, especially when commonly used alkylating drugs were applied.$^{17}$ However, in our study, response to BSF-2 did not correlate with the level of spontaneous $^3$H-TdR incorporation, but did correlate with therapeutic response.

Therefore, we conclude that myeloma cells decrease dependence on BSF-2 along with disease progression, and response to BSF-2 correlates with therapeutic response.

**ACKNOWLEDGMENT**

We thank Dr D. M. Mtasiwa for the critical review of this manuscript and Hiroko Sumida and Yumiko Ohto for excellent secretarial assistance.

**REFERENCES**


Decrease in BSF-2/IL-6 response in advanced cases of multiple myeloma

H Asaoku, M Kawano, K Iwato, O Tanabe, H Tanaka, T Hirano, T Kishimoto and A Kuramoto

http://www.bloodjournal.org/content/72/2/429.full.html

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml