Response Patterns of Purified Myeloma Cells to Hematopoietic Growth Factors

By Kenneth C. Anderson, Rebecca M. Jones, Chikao Morimoto, Pearl Leavitt, and Bruce A. Barut

Tumor cells were isolated from the bone marrow of seven patients with multiple myeloma and from the peripheral blood of three patients with plasma cell leukemia using Ficoll-Hypaque (FH) density sedimentation followed by immune rosette depletion of T, myeloid, monocytoïd, and natural killer (NK) cells. Enrichment to \( \geq 93\% \) plasma cells was confirmed with Wright's-Giemsa staining, with intracytoplasmic immunoglobulin staining, and with staining using monoclonal antibodies (MoAbs) directed at B, T, myeloid, monocytoïd, and myeloma antigens in indirect immunofluorescence assays. Myeloma cells neither proliferated nor secreted Ig in response to G/M-CSF, G-CSF, M-CSF, interleukin-1a (IL-1a), interleukin-1b (IL-1b), interleukin-2 (IL-2), or interleukin-4 (IL-4). Significant proliferation (SI \( \geq 3.0 \)) was induced by interleukin-6 (IL-6) in six of ten patients (SI of 31 and 43 in two cases); and to interleukin-3 (IL-3) and interleukin-5 (IL-5), independently, in two patients each. Peak proliferation to IL-5 or IL-6 and to IL-3 occurred in cells pulsed with \(^{3}H\) thymidine at 24 and 48 hours, respectively; and proliferation to combinations of factors did not exceed that noted to IL-6 alone; Ig secretion was not documented under any culture conditions. Three myeloma-derived cell lines similarly studied demonstrated variable responses. The heterogeneity in the in vitro responses of myeloma cells and derived cell lines to exogenous growth factors enhances our understanding of abnormal plasma cell growth and may yield insight into the pathophysiology of plasma cell dyscrasias.

A number of lymphokines that stimulate proliferation and differentiation of human B cells have now been described.\(^1\) These include low mol wt B-cell growth factor (LBCGF),\(^2,3\) high mol wt BCGF,\(^4\) B-cell stimulatory factor 1 (BSF-1)/interleukin-4 (IL-4),\(^5,6\) T-cell replacing factor (TRF)/BCGF II/interleukin-5 (IL-5),\(^7,8\) B-cell differentiation factor 1/BSF-2/interleukin-6 (IL-6),\(^9,10\) B-cell activating factor,\(^11\) pre-B-cell growth factor,\(^12\) B-cell inhibiting factor,\(^13\) interleukin-2 (IL-2),\(^14-20\) and gamma interferon.\(^21-23\) These factors may induce proliferation (IL-2, BSF-1/IL-4, TRF/BCGF II/IL-5) and Ig secretion (IL-2, BSF-2/IL-6) in activated B cells. In some cases molecular cloning of cDNA has facilitated further characterization of these growth factors (eg, the identification of BCGF II as TRF\(^9\) and BSF-2 as B2 interferon\(^24,25\) and hybridoma-myeloma growth factor).\(^26-28\) Further analysis of the structure and function of these lymphokines, coupled with ongoing delineation of the spectrum of the expression of the genes and receptors for these proteins, will further our understanding of normal B-cell activation and differentiation.

These lymphokines also appear to affect growth of transformed and malignant B cells.\(^29-39\) L-BCGF, for example, causes proliferation of hairy cells, B-cell non-Hodgkin's lymphomas, and B-cell acute lymphoblastic leukemias.\(^30-32\) Murine as well as human BCGF II can induce both proliferation and Ig secretion in a murine leukemia B-cell line BCL-1,\(^33-36\) BSF-2, a factor that triggers the terminal differentiation of normal B cells, also results in Ig secretion by a monoclonal human B-lymphoblastoid cell line, CESS.\(^37-39\) A proliferative response to BCGF by cells freshly isolated from a patient with B-chronic lymphocytic leukemia has also been described.\(^38\) Of great interest are the recent reports of Kawano et al postulating that myeloma may display an autocrine growth pattern based on the observations that these tumor cells express BSF-2 messenger RNA (mRNA), secrete BSF-2, express receptors for BSF-2, and proliferate in a specific manner to exogenous recombinant BSF-2.\(^39,40\) These in vitro studies suggest a possible pathophysiologic significance of lymphokines for in vivo tumor growth.

In the present report we have attempted to define the in vitro proliferation and Ig secretion response of myeloma cells and derived cell lines to recombinant lymphokine(s). Immune rosette depletion techniques were used to prepare homogeneous populations of myeloma cells, and the time course and magnitude of their responses to recombinant lymphokine(s) known to trigger activation and differentiation of normal B cells were characterized. Moreover, those cells that do respond to growth factors were examined for morphology, cell surface phenotype, as well as intracytoplasmic Ig (clg). Our studies demonstrate that some freshly purified homogeneous populations of myeloma cells may proliferate to IL-3, IL-5, and IL-6, further supporting the view that growth factors may play a role in the pathophysiology of plasma cell dyscrasias.

 Materials and Methods

Preparation of Myeloma-Enriched Populations

Myeloma cell-enriched populations were prepared from the bone marrow of seven patients with myeloma and from the peripheral blood of three patients with plasma cell leukemia using a method of immune rosette depletion.\(^41\) Myeloma mononuclear cells (MMCs) were isolated from myeloma bone marrow by Ficoll-Hypaque (FH) density sedimentation.\(^42\) MMCs (50 x 10\(^6\)/mL media) were next incubated for 30 minutes at 4\(^\circ\)C with excess concentrations (1:100) of anti-T3 (CD3) and T11 (CD2),\(^43\) anti-Mo(CD11) and Mo2(CD14),\(^44\) anti-NKH1,\(^45\) and anti-My9(CD33)\(^46\) monoclonal antibodies (MoAbs) to label T cells, monocytes, natural killer (NK) cells, and plasma cells, respectively. Treatment of MMCs with a saturating concentration of anti-CD19 MoAb (Becton Dickinson) for 10 minutes at 4\(^\circ\)C followed by extensive washing resulted in the depletion of B cells and natural killer cells. MMCs were next stained with rhodamine-conjugated anti-IgM (CD19, CD20, CD21), anti-CD14, and anti-CD3 (Becton Dickinson) antibodies. Finally, MMCs were stained with FITC-conjugated anti-CD38 (Becton Dickinson) and finally stained with fluorescein isothiocyanate-conjugated anti-CD56 (Becton Dickinson) antibodies. The resulting triple-stained MMCs were analyzed by flow cytometry to identify CD19+CD20+, CD14+, anti-IgM+, and CD56+ myeloma cells.

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cells and myeloid cells, respectively. These MoAbs were chosen to identify non-B lineage cells in bone marrow. Antibody-bound cells were depleted by the formation of a rosette with sheep erythrocytes coated with purified rabbit antinouse immunoglobulin as previously described, resulting in significant enrichment (93%) for myeloma cells in the rosette-negative fraction.

Characterization of Myeloma-Enriched Populations

The myeloma-enriched cell populations were characterized at the time of and at intervals after culture with growth and differentiation factors using Wright-Giemsa staining, clg staining, and staining with MoAbs directed at B, T, myeloid, and myeloma antigens in indirect immunofluorescence assays.

Intracytoplasmic Immunoglobulin Staining

Cells containing clg were enumerated by immunofluorescence with either anti-IgG, IgM, k, or a directly conjugated to fluorescein or rhodamine (DaKo Immunoglobulins), as previously described. These directly labeled reagents were tested on myeloma cells of known IgG, IgM, k, and a isotype and were found to be specific prior to use. Cells containing clg were enumerated using a Zeiss Fluorescent Microscope (Carl Zeiss, Inc., NY). At least 200 cells were counted per slide.

Phenotypic Analysis of Myeloma Cell-Enriched Populations and Myeloma Cell Lines

Plasma cell-enriched populations were examined using indirect immunofluorescence and flow cytometric analysis as previously described. Antibody-coated cells were enumerated by flow cytometry analysis using an EPICS V cell sorter (Coulter Electronics, Hialeah, FL). Monoclonal reagents used included the following: anti-B4(CD19), common acute lymphoblastic leukemia antigen (CALLA;CD10), B1(CD20), B2(CD21), B5, interleukin-2R (IL-2R;CD25), T10(CD38), and PCA-1, which are reactive with B cells at various stages of differentiation; with anti-T3 (CD3); with anti-Mo2(CD14), My9(CD33), and NKH1, reactive with monocyteid, myeloid, and NK cells, respectively. These reagents were directly conjugated to fluorescein or X directly conjugated to rhodamine (Bioran, Cambridge, MA) was also done on these supernatants.

Culture of Myeloma-Enriched Populations With Growth Factors

The IM-9, RPMI-8226, and U-266, myeloma-derived cell lines were cultured with hematopoietic growth factor(s) and proliferation and Ig secretion measured as described above.

RESULTS

Preparation of Myeloma-Enriched Populations

Myeloma cell-enriched populations were examined for morphology as well as clg and cell-surface phenotype. These populations had the morphology of mature plasma cells (Fig 1A) and also demonstrated the intracytoplasmic monotypic protein characteristic of the individual patient (Fig 1B). The cell-surface phenotype of myeloma-enriched populations, as defined by indirect immunofluorescence, is displayed in Table 1. Immune rosette depletion of T cells, monocytes, and myeloid cells resulted in 57% total residual T3-, Mo2-, and My9-staining cells, respectively. The T10 (CD38) antigen was most strongly expressed on a median of 64 (11% to 87%) of cells, whereas anti-PCA-1 reactivity was noted on a median of 47 (2% to 72%) of cells. The B4, CALLA, and B1 antigens were expressed on 2, 4, and 6 patients, respectively. Myeloma cells from a single patient (patient 6, Table 1) expressed little, if any, significant T10 and PCA-1 antigens but strongly expressed B1. Those cells with the highest percentage staining for the B-cell B4, CALLA, and B1 antigens (patient 4, Table 1) more weakly expressed T10 and PCA-1 plasma cell antigens. In contrast, those cells with the highest percentage reactivity with anti-T10 and anti-PCA-1 MoAbs (patient 10, Table 1) lacked significant B4, CALLA, or B1 expression.

Characterization of the Response of Purified Myeloma Cells to Growth Factors

Myeloma cell-enriched populations were cultured with growth factor(s), pulsed with [3H]TdR at 24 hours, and harvested 18 hours later. As can be seen in Table 2, the [3H]TdR incorporation was enhanced (93%) for myeloma cells cultured in media or with growth factors, with maximal proliferation of myeloma cells occurring at 10 U/mL. Cells were pulsed at the time of maximal incorporation of [3H]TdR, and harvested as described above.

Brmodoxymuridine staining was used as an additional measure of DNA synthesis. Before and after culture with medium or growth factor, cells were incubated with 10 μM BrdU (Sigma, St Louis) for 30 minutes at 37°C. Cytocentrifuge slides of the labeled cells were made. The cells were fixed in ethanol, immersed in 0.7 mol/L NaOH for two minutes, and incubated with anti-BrdU antibody (1 μg/sample; Becton Dickinson, Mountain View, CA) for 30 minutes. Cells were washed and stained with goat antinouse IgG fluorescein isothiocyanate (FITC) for 30 minutes. After washing, the cells were stained with propidium iodide (0.06 μg/μL). The proportion of cells that incorporated BrdU was determined by observing under fluorescence microscopy and counting at least 200 cells per slide. All counts were performed in duplicate.

Radioimmunoassay for IgG

Supernatants were harvested at various intervals after culture of myeloma cells with growth factor(s) and examined for secretion of IgG using a solid-phase radioimmunoassay (RIA) as previously described. Immunoelectrophoresis for kappa (k) or lambda (λ) light chains (Bioran, Cambridge, MA) was also done on these supernatants.

Culture of Myeloma Cell Lines with Growth Factors

The IM-9, RPMI-8226, and U-266, myeloma-derived cell lines were cultured with hematopoietic growth factor(s) and proliferation and Ig secretion measured as described above.
RESPONSE OF MYELOMA TO GROWTH FACTORS

Myeloma cell-enriched populations were prepared by immune rosette depletion of T cells, monocytes, NK cells, and myeloid cells from bone marrow and peripheral blood of patients with myeloma and plasma-cell leukemia, respectively. These populations had the morphology of mature plasma cells (Fig 1A) and demonstrated intracytoplasmic monotypic protein (Fig 1B). After three days of culture in medium, only rare clq-positive clusters of intermediate stage (pro) plasma cells with single nucleoli and a majority of nonviable cells were present (Fig 1C and D). Intracytoplasmic Ig-positive early plasmablasts and intermediate proplasma cells predominated after three days of culture with IL-3, with only rare mature plasma cells (Fig 1E and F).

Fig 1. Myeloma cell-enriched populations were prepared by immune rosette depletion of T cells, monocytes, NK cells, and myeloid cells from bone marrow and peripheral blood of patients with myeloma and plasma-cell leukemia, respectively. These populations had the morphology of mature plasma cells (Fig 1A) and demonstrated intracytoplasmic monotypic protein (Fig 1B). After three days of culture in medium, only rare clq-positive clusters of intermediate stage (pro) plasma cells with single nucleoli and a majority of nonviable cells were present (Fig 1C and D). Intracytoplasmic Ig-positive early plasmablasts and intermediate proplasma cells predominated after three days of culture with IL-3, with only rare mature plasma cells (Fig 1E and F).

uptake in the presence of medium alone was minimal and comparable (range 110 to 968 cpm). Significant proliferation, defined as a stimulation index (SI) > 3.0, did not occur over this interval to G/M CSF (Table 2) or to G-CSF, M-CSF, IL-1α, IL-1β, IL-2, and IL-4 (data not shown). Significant proliferation was noted in two patients each to IL-3 or to IL-5 alone. Six patients demonstrated significant proliferation to IL-6, and in two patients (patients 2 and 4, Table 2), the proliferative response to IL-6 was very pronounced (SI 31.5 and 45.3, respectively).

Once the pattern of response to single-growth factor was defined, combinations of factors were used to determine whether increased proliferation could be induced. In three cases (patients 3, 5, and 6, Table 3), the combination of IL-3 + IL-5 growth factors did result in significant proliferation, whereas either of these growth factors alone had no effect. In a single case (patient 4, Table 3), increased proliferation (SI 5.2) was demonstrated compared with that noted to IL-3 (SI 3.4) or IL-5 (SI 2.6) alone. In no case was proliferation to combinations of factors significantly greater than that noted to IL-6 alone. Finally, patients whose cells did not proliferate to single factors (patients 7 through 10, Table 3) were also unresponsive to combinations of factors.

The time course of proliferative response of purified myeloma cells was next examined. Growth factors were added at the initiation of culture, [3H]TdR pulses were done
at various intervals afterwards, and radioactivity counted 18 hours later. As can be seen, there was no proliferation noted to G/M CSF, confirming the lack of triggering of myeloma cells and also suggesting that few contaminating responsive cells remain. Although proliferation was documented in cells pulsed at day 0 and harvested 18 hours later, peak proliferation occurred when cells were pulsed with [3H]TdR at 24 hours to both IL-5 (SI 8.6) and IL-6 (SI 12.6) and was noted in cells pulsed at 48 hours to IL-3 (SI 7.3). Secretion of Ig did not occur during or after the proliferative response, even when cultures were extended out to 192 hours. More long-term cultures with repeated additions of growth factor(s) have not yet been attempted.

Characterization of the Ig Secretion Response of Purified Myeloma Cells to Growth Factor(s)

Myeloma cell-enriched populations cultured with growth factor(s) were also examined for secretion of Ig. The myelomas examined were of the following isotypes, as documented for secretion of Ig. The myeloma-enriched populations cultured with growth factors, revealed few positive cells (0.7%) after three days of culture in medium. However, the number of cells staining positive for BrdU was increased after culture with IL-3 (6.2% ± 0.5%) and, to a lesser extent, after culture with IL-5 (2.1% ± 0.5%) or IL-6 (1.6% ± 0.2%).

Before culture, frequent clusters of mature plasma cells and few nonviable cells were evident (Fig 1A). Only rare clusters of intermediate stage (pro) plasma cells with single nucleoli and a majority of nonviable cells were present after three days of culture with medium (Fig 1C). In contrast, early plasmablasts and intermediate proplasma cells predominated after three days of culture with IL-3, with only rare mature plasma cells (Fig 1E). After three days of culture with either IL-5 or IL-6, the majority of cells were nonviable, but rare clusters of early plasmablasts and intermediate proplasma cells were observed (not shown). Intracytoplasmic Ig staining revealed predominantly Ig-positive cells on day 0 (Fig 1B). Few (4.3%) Ig-positive cells

Table 1. Cell Surface Phenotype of Myeloma Cells

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<tr>
<th>Patient</th>
<th>B4</th>
<th>CALLA</th>
<th>B1</th>
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<th>PCA-1</th>
<th>T3</th>
<th>Mo2</th>
<th>My9</th>
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<td>3</td>
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<td>0</td>
<td>23</td>
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<td>81</td>
<td>71</td>
<td>2</td>
<td>3</td>
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</tbody>
</table>

* Mononuclear cells were isolated from the bone marrow of patients (1 through 7) with myeloma and from the peripheral blood of patients (8 through 10) with plasma-cell leukemia by Ficoll density sedimentation. Immune rosette depletion was used to deplete T cells, monocytes, NK cells, and myeloid cells. The resulting myeloma-enriched populations were then examined using indirect immunofluorescence and flow cytometric analysis.

Table 2. Response of Myeloma Cells to Single-Growth Factors*

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>None</th>
<th>G/M CSF</th>
<th>IL-3</th>
<th>IL-5</th>
<th>IL-6</th>
<th>IL-6</th>
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<td>G/M TdR</td>
<td>G/M TdR</td>
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<td>G/M TdR</td>
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</tr>
<tr>
<td>1</td>
<td>282 ± 20</td>
<td>314 ± 24</td>
<td>1.1</td>
<td>1,799 ± 25</td>
<td>6.4</td>
<td>960 ± 26</td>
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<tr>
<td>2</td>
<td>968 ± 20</td>
<td>1,375 ± 209</td>
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<td>2,282 ± 141</td>
<td>2.4</td>
<td>26,870 ± 1077</td>
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<tr>
<td>3</td>
<td>205 ± 73</td>
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<td>110 ± 47</td>
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<td>1.7</td>
<td>370 ± 140</td>
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<tr>
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<td>1.7</td>
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<td>860 ± 37</td>
<td>1.2</td>
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<td>10</td>
<td>219 ± 38</td>
<td>273 ± 87</td>
<td>1.3</td>
<td>327 ± 92</td>
<td>1.5</td>
<td>323 ± 67</td>
</tr>
</tbody>
</table>

* Purified myeloma cells were suspended at 5 x 10^6/mL in RPMI/10% FCS, and 100-μL aliquots were dispensed in 96-well round-bottomed tissue culture plates with 100 μL of either media or growth factor.

† Cells were pulsed at 24 hours with 0.2 μCi/well [3H]TdR, were harvested onto glass filters, and were counted on a scintillation counter 18 hours later.

‡ Si = [3H]TdR uptake of sample/[3H]TdR uptake of control.
remained after three days of culture with medium (Fig 1D) or with IL-6 (3.3%), but relatively increased numbers of clg-positive cells were present after culture with IL-3 (14%); (Fig 1F) and IL-5 (12%).

Sufficient cells were available after peak response to IL-6 in two cases (patients 2 and 4) for phenotypic characterization. Cells from patient 2 before and after culture with IL-6 were B4-CALLA±BI±PCA-I (Table 1). The cell surface phenotype of patient 4 before and after culture with IL-6 remained B4+CALLA±B1±PCA-I (Table 1, Fig 2A and B, respectively). Cells from four of the five patients that either did not respond or minimally proliferated to IL-6 (patients 3, 7, 9, and 10) have the B4-CALLA-Bl-PCA-l (Table 1, Fig IF) and IL-5 (12%).

**Characterization of Myeloma Cell Lines for Cell-Surface Phenotype and Response to Growth Factors**

Three myeloma cell lines (IM-9, RPMI-8226, and U-266) were examined for their cell-surface phenotype and for response to growth factor(s). The cell-surface phenotypes, as defined using indirect immunofluorescence techniques, are displayed in Table 5. All three cell lines demonstrate only weak staining with cell surface Ig, but all are of B lineage by virtue of their expression of B and/or plasma cell antigens. The majority (83%) of IM-9 cells strongly express the B-cell–restricted B4 and B2 antigens; and only 36% and 10% express the B1 and B5 B-cell–restricted antigens; respectively; nearly one half of cells express the plasma cell antigen PCA-I and <10% express cell-surface T10. RPMI-8226 and U-266 cells, in contrast, express B-cell–restricted antigens weakly, if at all, and strongly express T10 and PCA-l antigens on 47% to 95% and 87% to 97% of cells, respectively.

The three myeloma cell lines were next examined for their proliferation and Ig secretion in response to growth factors (Table 6). A myeloma-derived line, IM-9, did not proliferate to growth factor(s) but did demonstrate increased IgG secretion to IL-3 alone (80 ng) and markedly increased IgG secretion (≥500 ng) to either IL-5 or IL-6 alone and to combinations of growth factors. The proliferation of U-266 cells to single-growth factors was not significant (SI = 1.7); however, proliferation was markedly augmented (SI up to 7.5) to combinations of factors. No secretion of IgG was noted, even to combinations that did result in a proliferative response. Finally, RPMI-8226 neither demonstrated significant proliferation nor secreted IgG to any single or combination of growth factors. Immunoelectrophoresis did not reveal any light chain secretion by RPMI-8226 under any culture conditions.

**DISCUSSION**

In this report we have examined the response of purified myeloma cells to hematopoietic growth factor(s). No significant proliferation was noted to G/M-CSF, G-CSF, M-CSF, IL-1α, IL-1β, IL-2, or IL-4. In contrast, significant proliferation of cells pulsed with [3H]Tdr at 24 hours and harvested 18 hours later was noted in two patients each to IL-3 or IL-5 alone. Of particular note was the proliferation induced by IL-6 in six of ten patients, with stimulation indices of 31 and 43 in two cases. Although the proliferative response to IL-3 and IL-5 appeared to be additive in coculture experiments, in no case was proliferation to combinations of factors signifi-
Fig 2. Cell-surface phenotype of myeloma cells. Myeloma cell-enriched populations were prepared by immune rosette depletion of T cells, monocytes, NK cells, and myeloid cells from bone marrow and peripheral blood of patients with myeloma and plasma-cell leukemia, respectively, and then cultured in vitro with growth factor(s). The cell-surface phenotype of patient 4, whose cells did proliferate to IL-6, remained B4 + CALLA ± B1 + PCA-1 ± before and after culture with IL-6 (Fig 2A and B). Cells that did not proliferate to IL-6 (patient 10) had the B4-CALLA-B1-PCA-1 + cell-surface phenotype (Fig 2C).

In our studies the enrichment technique used was immune rosette depletion of T3+ and T11+ T cells, Mol+ and Mo2+ monocytes, NKH1+ NK cells, and My9+ myeloid cells. Such purification techniques are critical, since T cells, myeloid cells, macrophages, and hematopoietic stem cells may also respond to exogenous growth factors and that myeloma-derived cell lines may not always similarly respond. These observations may give insight into both the biology of abnormal plasma-cell growth and the pathophysiology of plasma cell dyscrasias.

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Table 5. Cell-Surface Phenotype of Myeloma-Derived Cell Lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>la</th>
<th>CALLA</th>
<th>B4</th>
<th>B1</th>
<th>B2</th>
<th>B5</th>
<th>Tac</th>
<th>T10</th>
<th>PCA-1</th>
<th>IgM</th>
<th>IgG</th>
<th>IgD</th>
<th>K</th>
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*FH-viable cells were examined using indirect immunofluorescence and flow cytometric analysis.
responsive to growth factor(s). Our data suggest that those myeloma cells that proliferated to the greatest extent to IL-6 (patients 2 and 4) have an earlier B-cell phenotype (B4±CALLA±B1±PCA-1±) than those cells that either did not (patients 6 through 10) or weakly (patient 3) respond (B4-CALLA-B1±PCA-1±). There is no evidence from the current data to suggest that those cells that proliferated to IL-6 are in fact of a different phenotype than those at the initiation of culture (eg, that only a subset of cells respond to this stimulus). Nonetheless, further experiments need to be done to determine which cell(s) within the myeloma clone can be triggered by growth factors. Recently described techniques for the culture of myeloma cells,67 coupled with the above-described responses to lymphokines and with newer techniques of multiple fluorochrome immunofluorescence analysis that have proven useful for the study of both normal and neoplastic B cells,75,76 may permit the phenotypic definition of those myeloma cells that have the capacity to proliferate in response to growth factor or those with self-renewal capacity. Phenotypic purification of such cells using these techniques may both facilitate their biological characterization in vitro and permit enhanced understanding of aberrant B-cell differentiation in vivo.

The responses of freshly isolated and purified myeloma cells to exogenous growth factors do not mirror the pattern of responses noted in three myeloma-derived cell lines. For example, fresh myeloma cells proliferated without secreting Ig to IL-6; myeloma-derived cell lines also proliferated without secreting Ig, or secreted Ig without proliferating, or did neither. Indeed, the effect of BSF-2 on other chronic lymphocytic leukemia cell lines, such as BCL1 and CESS, has been to enhance Ig secretion without proliferation.11,37 This lymphokine has no demonstrated growth activity but does cause the final maturation of normal B cells to high rates of Ig secretion.11,12 These varied effects of growth factors on freshly isolated tumor cells, tumor cell lines, and normal B cells point out the necessity of studying freshly isolated tumor cells, since response patterns seen in either cell lines or normal cells do not predict for tumor-cell responsiveness. Further study of the mechanisms accounting for these differences in response patterns may also greatly enhance our understanding of aberrant B-cell differentiation.

The proliferative response of myeloma cells to exogenous recombinant human BSF-2 has recently been reported by Kawano et al,38 and Asaoku et al.40 Based upon their studies, they postulate that autocrine stimulation may be a mechanism of transformation in multiple myeloma. Our data are consistent with this hypothesis but further suggest that IL-3 and IL-5 may also trigger DNA synthesis by myeloma cells. These observations suggest that specific inhibitors of growth factors could be potentially useful for in vivo therapy of plasma cell dyscrasias; however, growth factor antagonists may also adversely affect hematopoietic stem cells, thereby limiting their clinical use. In an alternative treatment application, the proliferative response of myeloma cells to growth factors could be used to improve in vitro purging of myeloma cells from autologous marrow prior to transplantation. Specifically, myeloma cells within autologous marrow could be induced to proliferate by growth factor in vitro and a cell-cycle chemotherapeutic agent then be used to specifi-
cally kill proliferating cells. This proposed strategy may permit the use of high doses of systemic chemotherapy and total body radiation and subsequent transplantation of tumor-free autologous marrow. Thus response patterns to exogenous growth factors may not only augment our understanding of the biology of plasma-cell neoplasms but may also suggest new therapeutic options.

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Response patterns of purified myeloma cells to hematopoietic growth factors

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