Interleukin-6 Gene Expression in Multiple Myeloma: A Characteristic of Immature Tumor Cells

By Hiroyuki Hata, Huqing Xiao, Maria Teresa Petrucci, Jeff Woodliff, Ruixin Chang, and Joshua Epstein

Interleukin-6 (IL-6) has been suggested to play a major role in multiple myeloma. To investigate the source and target cells of IL-6 activity in multiple myeloma, expression of the cytokine and its receptor genes by myeloma plasma cells was studied. Tumor cells were sorted from bone marrow aspirates of myeloma patients using 4-parameter gating. Myeloma cells were identified as CD38high CD45negative-intermediate and by their light-scatter characteristics. Sorted cells contained only myeloma plasma cells. No contaminating cells were present as determined morphologically, by monoclonal cytoplasmic Ig analysis, and by polymerase chain reaction (PCR) amplification of marker genes. Myeloma cells from 45% of patients expressed IL-6; IL-6 receptor transcripts were found in 68% of the specimens. IL-6 gene expression correlated with expression of the IL-6 receptor gene (P < .005). Correlations observed between the expression of CD45, a protein tyrosine phosphatase expressed by B lymphocytes but not by plasma cells, and the expression of the IL-6 and IL-6–receptor genes (P < .0002 and P < .005, respectively) suggest that an autocrine IL-6 loop is functioning in myeloma in preplasma cells.

The maturation stage of B-cell neoplasias vary from pre-B-cell leukemia to multiple myeloma, a hematologic malignancy characterized with mature plasma cell morphology and function. Whereas the recognizable tumor cells in myeloma are the most mature B cells, early lymphoid cells are involved in the disease and probably represent the proliferative, preplasma cells compartment. The apparent involvement of a common early progenitor in the B-cell neoplasias and possibly in all hematologic malignancies underscores the importance of defining the factors that determine the phenotypic presentation of the neoplasms.

Recently, studies have shown a role of lymphokines in the regulation of proliferation and differentiation of the hematopoietic system cells. The multifunctional interleukin-6 (IL-6) has been proposed as the major myeloma cell growth factor. Whereas some studies have suggested that IL-6 functions as an autocrine growth factor, others have favored a paracrine growth stimulation mechanism. IL-6 and IL-3 function in concert to promote proliferation and differentiation of early B cells in the bone marrow (BM) and blood from myeloma patients into myeloma plasma cells. Apparently, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-1α modulate the proliferative response of myeloma cells to IL-6.3,14 Tumor necrosis factor α (TNF-α), which is produced by accessory cells, stimulates the production of IL-6 and other lymphokines by a variety of hematopoietic and other cells.

Whether IL-6 stimulation of myeloma is autocrine or paracrine is important clinically. For instance, neutralizing anti–IL-6 antibody therapy could present an attractive option for a paracrine-stimulated IL-6–dependent tumor. However, such therapy may not be effective against an autocrine IL-6–dependent tumor, especially if IL-6 does not need to exit the cell to be active. In such cases, IL-6 receptor targeting therapy, such as recently reported, could be an attractive alternative.

Investigations aimed at clarifying the sources and activities of IL-6 in myeloma have been impeded by the difficulty of obtaining purified tumor cells from myeloma patients in adequate numbers. A modification to a recently developed method was used to purify myeloma cells from BM aspirates of patients with plasma cell myeloma. Two myeloma plasma cell populations, differing only in the expression of some surface antigens, coexisted in most patients. Expression of IL-6 and its receptor genes by highly purified myeloma cells from 22 patients was determined.

Materials and Methods

Patients. Twenty-two patients with multiple myeloma, 6 women and 16 men, 36 to 67 years of age (median 52 years), at all treatment stages were studied. Their relevant clinical information is summarized in Table 1. All had a single monoclonal myeloma cell population as determined by heavy chain Ig gene rearrangements. The only selection criteria for study were marrow myeloma cell populations of greater than 5% and a sufficiently high recovery of purified myeloma cells.

Myeloma cell preparation. Heparinized BM aspirates were obtained during routine visits to the clinic, as required by treatment protocols. Signed consent forms were obtained before study. Myeloma cells were purified using a modification of a recently described method. After density separation (Histopaque; Sigma, St Louis, MO), light-density cells were reacted for 30 minutes at 4°C with a mixture of phycoerythrin-conjugated anti-CD38 and fluorescein isothiocyanate-conjugated (FITC) anti-CD45 monoclonal antibodies (MoAbs) (Becton Dickinson Immunocytometry, San Jose, CA) and washed twice with phosphate-buffered saline (PBS). Cells showing high-intensity CD38 and low-to-intermediate CD45 fluorescence with negative-to-intermediate orthogonal and intermediate-to-high forward light-scatter profile were sorted using a FACStar Plus flow cytometer/cell sorter (Becton Dickinson) at a flow rate of 1,000 to 1,200 events per second. Cytospin slides were pre-

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pared and stained with May-Gr"unwald Giemsa stain for morphologic evaluation. Ethanol-fixed slides were reacted with F(ab)$_2$ fragments of antihuman Ig light-chains antibodies for evaluation of monotypic cytoplasmic Ig content. Nonmyeloma cells (lymphocytes, monocytes, and myeloid cells) were sorted into a separate tube and were used as controls.

Monocytes. Mononuclear cells separated from 10 mL of blood by density centrifugation as described above, were layered over 5 mL Percoll (49.2%, Sigma) and centrifuged at 1,130g for 25 minutes. The cells from the upper band were recovered and washed with PBS. The proportion of monocytes was determined by morphologic evaluation using May-Gr"unwald Giemsa-stained cytospin slides and by flow cytometry using MoAb to Bence-Jones protein only, NS, non-secerning patients.

Morphology. Mononuclear cells separated from 10 mL of blood by density centrifugation as described above, were layered over 5 mL Percoll (49.2%, Sigma) and centrifuged at 1,130g for 25 minutes. The cells from the upper band were recovered and washed with PBS. The proportion of monocytes was determined by morphologic evaluation using May-Gr"unwald Giemsa-stained cytospin slides and by flow cytometry using MoAb to CD14 (Becton Dickinson).

Gene expression. To overcome the obstacles presented by the relatively small numbers of purified cells as well as the anticipated low levels of IL-6 gene expression by myeloma cells, the polymerase chain reaction (PCR) technique for detection of specific mRNA was used. Total cellular RNA was isolated using a microadaptation of the guanidinium thiocyanate/cesium chloride procedure. Briefly, cell pellets containing 1 to 4 x 10$^6$ purified cells were resuspended in 100 mL of 4 mol/L guanidinium isothiocyanate, vortexed for 1 to 2 minutes, layered over a 100 mL cushion of 5.7 mol/L cesium chloride in a polycarbonate tube and centrifuged at 80,000 rpm for 2 hours at 20°C in a refrigerated tabletop ultracentrifuge (TL-100; Beckman, Fullerton, CA). The RNA pellet was resuspended in 50 mL diethyl pyrocarbonate (DEPC) water, precipitated with ethanol, dried, and resuspended in 10 mL cDNA-synthesizing solution. First-strand cDNA was synthesized using Moloney murine leukemia virus (MMLV) reverse transcriptase and oligo dT (Boehringer Mannheim, Indianapolis, IN) for 1 hour at 42°C. After inactivation of the reverse transcriptase at 95°C for 10 minutes, 20 mL water was added to a final volume of 30 mL, and a 1- to 5-mL aliquot was used as template for 35-cycle amplification by PCR with cytokine-specific primers (Table 2). Temperature cycling was 1 minute at 94°C, 2 minutes at 60°C, followed by 3 minutes at 72°C. Aliquots of 10 mL of the PCR product were analyzed by electrophoresis on 2% agarose gel containing 0.5 g/mL ethidium bromide. In single-cell PCR experiments, the total amount of cDNA left after &actin amplification was used for amplification with IL-6 primers, and the total amplification product was analyzed by ethidium bromide gel electrophoresis. In several experiments the amplification products were transferred to a membrane, hybridized to the appropriate $^{32}$P-labeled probe, and processed autoradiographically, to further confirm their identity and the specificity of the PCR reaction.

RESULTS

Some samples contained as few as 5% myeloma cells as determined by morphology or by monotypic cytoplasmic Ig content analyzed by flow cytometry. Nevertheless, the myeloma cell preparations consisted of pure myeloma plasma cells (Fig 1A). CD38 and CD45 profiles of two representative samples are presented in Figs 1, B and C. The monocyte-enriched preparations contained greater than 96% monocytes by morphology and CD14 expression, with occasional lymphoid cells.

Cytokine gene expression was studied by PCR. Figure 2 shows examples of PCR analysis using primers for IL-6, IL-6-receptor (IL-6-R), IL-1$, and TNF-$(Table 2). Primers for the $\beta$-actin gene were used as control for cDNA integrity, for the presence of cells in single-cell PCR experiments, and for PCR. IL-6 mRNA was detected in purified myeloma cells from 10 of 22 patients (45%). IL-6-R mRNA was detected in the 10 patients in whom IL-6 gene expression was found, and in five additional patients in whom IL-6 transcripts were not detected. Myeloma cells from seven patients did not express the IL-6-R gene. Thus, the receptor mRNA was detected in every instance where IL-6 mRNA was expressed.

TNF-$( mRNA was detected in 8 of 21 patients tested. IL-1$ transcripts were not detected in myeloma cell preparations from any of the patients studied. TNF-$( gene expression did not correlate with expression of the IL-6 and IL-6-R genes. The results for IL-6, IL-6-R, TNF-$(, and IL-1$ expression by myeloma cells are summarized in Table 3.

PCR is capable of detecting the presence of even one contaminating cell among 10$^5$ cells. Although morphologically 100% of the cells were myeloma plasma cells, this high sensitivity of the PCR mandated the use of controls to assure that the results obtained using this procedure represented gene expression by myeloma cells and not the phenotype of rare contaminating cells. Because neither immunocytochemical staining, enzyme-linked immunosyotus (ELISSPOT), or in situ hybridization (S.H., personal communication, May 1992; and M.T.P. and J.E., data not shown) techniques

<table>
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<tr>
<th>Table 1. Patient Characteristics</th>
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<tr>
<td>Variable</td>
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<td>Stage*</td>
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<tr>
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<td>II</td>
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<tr>
<td>NS</td>
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<tr>
<td>Prior treatment</td>
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<tr>
<td>Status†</td>
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Abbreviations: BJP, Bence-Jones protein only, NS, non-secerning patients.

* Stage according to the Durie-Salmon staging.
† Disease status at time of study.
§ Therapy-resistant relapse.

<table>
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<th>Table 2. Primers for PCR</th>
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<tr>
<td>Gene</td>
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<tr>
<td>IL-6</td>
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<tr>
<td>IL-1$</td>
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<tr>
<td>$\beta$-Actin</td>
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<td>IL-6-R</td>
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<tr>
<td>TNF-$(</td>
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Fig 1. (A) Sorted myeloma cells. Myeloma cells were identified as CD38$^{hi}$CD45$^{lo}$ and by light-scatter properties. Sorted cells contained only myeloma cells by morphology, monoclonal clg content and as determined by PCR (see Results). (B and C) CD38/CD45 expression by myeloma cells. CD38$^{hi}$CD45$^{lo}$ (B) and CD38$^{lo}$CD45$^{int}$ myeloma cells (C) are boxed. Coordinates represent fluorescence intensities (channel number) on a logarithmic scale. Note the presence of a small number of CD38$^{lo}$CD45$^{hi}$ cells in (B).

were capable of detecting the cytokine or IL-6 gene transcripts in myeloma cells, a different approach had to be used to ascertain purity of the myeloma cell preparations. The patterns of cytokine gene expression by purified myeloma cells were compared with those of monocyte-enriched preparations and of sorted nonmyeloma cells from the same patients, studied simultaneously. Results from several such experiments are presented in Fig 2 and in Table 4. Whereas all control cell preparations expressed IL-1β and TNF-α genes, none of the myeloma cell samples contained IL-1β gene transcripts, and only eight expressed TNF-α. In addition, while control cells from patients 2, 5, and 6 in Table 4 expressed IL-6, no IL-6 mRNA was detected in the purified myeloma cells; the opposite was observed for IL-6–R expression for patients 1 through 4. These clear and consistent differences in IL-1β gene expression between the myeloma and control cell populations, as well as additional differences in expression of the IL-6, IL-6–R, and TNF-α genes, prove the high degree of purity of the myeloma cell preparations.

Expression of the IL-6 gene by myeloma cells is suggestive of an autocrine IL-6 loop. Unless one accepts the possible existence of exclusively autocrine and paracrine forms of the disease, the variation in IL-6 expression by myeloma cells among different patients probably reflects tumor-cell heterogeneity. In the normal immune system, IL-6 functions as a differentiation-promoting factor, inducing maturation of B cells into Ig-producing cells. If IL-6 plays a similar role in myeloma, it would be plausible to assume an autocrine IL-6 loop to be functional in less differentiated tumor
Myeloma Cells

Control

1 - β-actin
2 - IL-6
3 - IL-6-R
4 - TNF-α
5 - IL-1β
6 - Size Markers

Fig 2. Cytokine gene expression by purified myeloma and control cells. Total RNA was extracted from 2 × 10⁶ sorted myeloma cells and from monocytes or sorted nonmyeloma cells (controls). cDNA synthesized and presence of β-actin, IL-6, IL-6-receptor, TNF-α, and IL-1β gene transcripts determined by PCR. Size markers (sizes in basepairs are given) were run in lane 6.

cells. Thus, the observed heterogeneity in IL-6 expression among patients could reflect differences in myeloma cell maturation.

Expression of CD45 by malignant B cells and shifts in CD45 isoform expression are indicators of the level of maturation of neoplastic B cells. Therefore, expression of the IL-6 and IL-6-R genes was compared with the expression of CD45, also heterogeneously expressed on myeloma cells (Figs 1A, 1B, 3, and 4). CD45 expression data were collected at the time of cell sorting. Myeloma plasma cells from 10 patients expressed CD45; of these, 9 also expressed the IL-6 gene. In contrast, only 1 of 12 CD45⁺ cell preparations showed IL-6 gene expression. The correlation between CD45 and IL-6 expression was highly significant (P < .0002, Table 5).

The observed association between CD45 and IL-6 expression was further investigated in four patients who had both CD45-expressing as well as CD45⁻ myeloma-cell subpopulations (Fig 5). Both cell populations consisted exclusively of monoclonal clg-containing plasma cells. Only purified CD45⁺ myeloma cells from these patients expressed the IL-6 gene, confirming the relationship between IL-6 and CD45 expression. These results also add support to the purity of the sorted myeloma cell preparations. Expression of CD45 also correlated with IL-6-R expression (P < .005, Table 5). No relationship was apparent between CD45, IL-6 or IL-6-R expression and clinical parameters such as stage and marrow plasmacytosis.

To further investigate the association between CD45 and IL-6 expression, individual CD45⁻ and CD45⁺ myeloma cells were sorted using the Automatic Cell Deposition Unit (Becton Dickinson) attachment. RNA extracted, and IL-6 gene expression studied. β-Actin was used as control for cell presence, cDNA integrity, and for PCR. β-Actin mRNA was detected in 16 of the 20 cell preparations. Of the 16, IL-6 transcripts were detected by ethidium bromide fluorescence only in CD45⁺ cells (Table 6).

Table 3. Expression of IL-6, IL-6-R, IL-1β, and TNF-α by Myeloma Plasma Cells

<table>
<thead>
<tr>
<th></th>
<th>IL-6</th>
<th>IL-6-R</th>
<th>IL-1β</th>
<th>TNF-α</th>
<th>N</th>
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<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>+</td>
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<td>-</td>
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<td>5</td>
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Table 4. Cytokine Gene Expression by Myeloma and Nonmyeloma Cells

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<tr>
<th>Patient</th>
<th>Cells</th>
<th>IL-6</th>
<th>IL-6-R</th>
<th>IL-1β</th>
<th>TNF-α</th>
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<tbody>
<tr>
<td>1</td>
<td>Plasma cells</td>
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<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Monocytes*</td>
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<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Plasma cells</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Monocytes</td>
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<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>5</td>
<td>Plasma cells</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Nonplasma cells</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</table>

* Cell preparations containing >95% monocytes.
† Cells sorted with the sort windows set to include all cells except plasma cells.

Fig 3. Between-patient heterogeneity of CD45 expression by myeloma cells. Histograms from two patients are shown. The myeloma cells from one patient were CD45⁻ (histogram a), from the other CD45intermediate (histogram b). Histograms c represent non-myeloma cells expressing high levels of CD45 from these patients, for comparison. Abscissa: intensity of CD45-associated fluorescence (log scale). Ordinate: relative cell number.
A bioassay was used to test whether myeloma cells that express the IL-6 gene also produce IL-6 protein product. Purified myeloma cells, $2 \times 10^5$, from nine patients were cultured in 100 \( \mu \)L media for 48 to 72 hours, after which the medium was filtered through a 0.22-\( \mu \)m pore-size membrane and the concentration of IL-6 determined using the IL-6-dependent B9 murine plasmacytoma cell line. IL-6 standard was a kind gift from Interpharm Laboratories (Israel). Only the five myeloma cell preparations with IL-6 mRNA expression produced IL-6, detected at concentrations of 0.5 to 12.8 pg/mL (median $= 1.16$ pg/mL).

**DISCUSSION**

The controversy surrounding the mechanism of IL-6 activity in myeloma reflects, in part, the difficulty in obtaining myeloma cells in sufficient quantities and of an adequate degree of purity to allow application of sensitive analytical methods. While enrichment of myeloma cells could be accomplished by physical, immunologic, and immuno-magnetic bead-based and panning methods, only cell sorting provided the high degree of selectivity needed for obtaining myeloma cells of a sufficiently high level of purity. The absence of contaminating cells was sought by adjusting instrument settings and sort windows to exclude all cells with marginal properties. As a result, collected cells represented only 20% to 50% of those selected, thus limiting the quantity of cells available for study and imposing the use of PCR as the most feasible method for studying cytokine and receptor gene expression.

Use of PCR in turn necessitated using strict controls to guarantee the validity of the results. The high sensitivity of PCR mandated negation of the possibility that the observed phenotype resulted from cosorted myeloma and nonmyeloma cells. The inability of less sensitive techniques to detect IL-6 gene expression by individual myeloma cells necessitated a different approach to be taken. This was accomplished by comparing expression of IL-6, IL-6-R, IL-1\( \beta \), and TNF-\( \alpha \) genes by myeloma cells with their expression by monocyte-enriched and by sorted nonmyeloma cell preparations. If one were to attribute the PCR results to rare contaminating cells, the following would imply: (1) contamination by IL-6-expressing nonmyeloma cells occurred only in the 45% of patients in whose myeloma cells IL-6 transcripts were detected (Table 3); (2) contamination with IL-6+ cells consistently occurred in patients with CD45-expressing tumor cells, whereas both cell populations expressed IL-6–R (lane 3).
pressing myeloma cells but only rarely in CD45- myeloma cells (9 of 10 v 1 of 11, respectively, Table 5); (3) contamination in all patients was only by IL-1β-, never by IL-1β+ nonmyeloma cells; (4) even in patients with coexisting CD45- and CD45+ myeloma cell subpopulations, only the sorted CD45+ subpopulations were contaminated with IL-1β- IL-6+ nonmyeloma cells; (5) the majority of CD45+ myeloma cells studied were, in fact, nonmyeloma cells (Table 6); and this is not compatible with morphologic and clg content evaluation of the sorted myeloma cells (Fig 1); and (6) sorted myeloma cells were preferentially contaminated by nonmyeloma cells while the reverse did not occur, and in Table 4, nonmyeloma cells of patients 1 through 4 were not contaminated with IL-6-R-expressing myeloma cells. Thus, it was concluded that the observed differences between these cell preparations in the patterns of cytokine and receptor-gene expression were proof of the purity of the myeloma cell preparations and attested to the validity of the PCR-derived data.

IL-6 expression by myeloma plasma cells correlated strongly with a CD45+ phenotype. Expression of CD45 on the surface of malignant B cells and shifts in CD45 isoform expression are indicators of the level of maturation of neoplastic B cells. Heterogeneity in CD45 expression by myeloma cells among different patients, observed in this study, likely reflects differences in the degree of maturation of the tumor cells. Indeed, CD45+ but not CD45- myeloma cells coexpressed the B-cell antigen CD19 (data not shown). In the majority (88%) of patients with CD45+ myeloma, small numbers of CD45intermediate clg-containing myeloma cells coexisted (eg, Fig 1B). In these, the levels of CD19 expression by the myeloma cells declined with the decrease in the level of CD45 expression, indicating a maturation process or a phenotypic shift to a more immature, possibly more aggressive disease. In four of these patients, sufficient numbers of cells from each myeloma cell subpopulation were purified for study; only the tumor cells displaying the less mature CD45+ phenotype expressed IL-6, underscoring the correlation between the two parameters.

A central role for IL-6 in myeloma has been proposed by several investigators. The current finding of IL-6 expression by freshly isolated myeloma cells displaying a less mature phenotype, although not evident, strongly supports the possibility of an autocrine IL-6 loop in multiple myeloma. Further support for this can be found in the close association between expression of IL-6 and IL-6-R genes (P < .005, Fisher's exact test). This association as well as production of IL-6 by purified CD45-expressing myeloma cells tends to discount the possibility that regulatory processes at the posttranscriptional level prevent translation of IL-6 as well as IL-6-R mRNA into the active IL-6 molecule and its receptor. It would appear that only minuscule amounts of IL-6 are required for maintaining an autocrine loop, as was reported for the myeloma cell line U266. This low level of expression could account for the difficulty in detecting IL-6 production in myeloma cells using even sensitive immunologic and immunocytochemical methods. The reported inhibition of proliferation of myeloma cell lines in which IL-6 production could not be detected by antisense to IL-6 underscores the likelihood of an autocrine IL-6 stimulation loop in multiple myeloma even when IL-6 expression cannot be detected with available methods other than PCR and a sensitive bioassay.

Both the CD45- and CD45+ myeloma cells used in this study were morphologically and phenotypically myeloma plasma cells. Both cell populations were nonproliferative, did not respond to exogenous IL-6, and likely are not the target cells for IL-6 activity. Rather, CD45+ myeloma plasma cells represent an intermediate stage in B-cell maturation. As such, while expressing plasma cell morphology and function (lg secretion), they are still in the process of losing the characteristics of B cells, such as CD19 and CD45, and also IL-6 and IL-6-R expression. This would imply that an autocrine IL-6 loop is functional in an even earlier, as yet unrecognized, tumor cell of B-cell morphology and phenotype (preplasma cell). Circulating monoclonal B cells found in patients with multiple myeloma and Waldenstrom’s macroglobulinemia could represent such preplasma cells. It is significant that these circulating cells have normal, albeit polyclonal counterparts, because this lends support to the hypothesis that the central role of IL-6 in myeloma is not different from the cytokine’s role in normal B-cell development.

IL-6 could provide a proliferative stimulus to myeloma progenitor cells, as has been suggested. Alternatively, akin to its role in normal B-cell development, the cytokine could act as a differentiation factor, facilitating maturation of premyeloma B cells into Ig-secreting myeloma plasma cells, without direct growth-stimulating activity. As a third possibility, IL-6 could affect both proliferation and differentiation. Existence of an autocrine IL-6 loop in premyeloma B cells, as the data suggest, would imply that these cells are constantly stimulated. The fact that a largely expanded preplasma cell compartment in myeloma patients has not been identified may suggest that the rate of preplasma cell proliferation is matched or probably exceeded by the rate of differentiation, or that the proliferative, preplasma cell population is maintained at a constant size. Under the latter scenario, cell proliferation could be regulated, possibly through a “feedback” loop, so as to compensate for the “loss” of cells to IL-6-mediated differentiation, without expansion of the proliferative pool. In this case, IL-6 would directly affect differentiation and indirectly, by allowing expansion to take place, also proliferation.

CD45 is a tyrosine phosphatase. It functions in receptor-mediated transmembrane signal transduction associated with tyrosine phosphorylation. Inhibition of CD45 activity by mutation or with MoAbs inhibits, among other pathways, B-cell activation, proliferation, and differentiation. One could speculate that loss of CD45 expression with normal or malignant B-cell maturation into normal or myeloma plasma cells is compatible with the loss of response to activating signals that accompanies terminal differentiation. In this context, the close association seen between IL-6, IL-6-R, and CD45 expression is particularly interesting. In addition to indicating the possible operation of an autocrine IL-6 loop in preplasma cells, it may suggest a
role for CD45 in IL-6/IL-6–R complex-mediated signal transduction.

In contrast to a previous report, IL-1β expression was not detected in myeloma cells of the 22 patients studied, whereas IL-1β transcripts were present in all control cell preparations. This discrepancy likely reflects differences in myeloma cell purity, which was greater than 95% using a physical/immunologic method compared with 100% reported here.

The data presented here strongly suggest the existence of an autocrine IL-6 loop in multiple myeloma; that the autocrine IL-6 loop is functional in preplasma cells; and that the recognizable myeloma cells, of plasma cell morphology and function, are not the target for IL-6 activity. Ongoing clinical trials with IL-6–neutralizing antibodies and work with IL-6–dependent and –independent cell lines as well as with agents such as dexamethasone that regulate IL-6 and IL-6–R gene expression, could potentially help solve the IL-6 enigma.

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