A Review of the Cytokine Network in Multiple Myeloma

Diagnostic, Prognostic, and Therapeutic Implications

Vito Michele Lauta, M.D.

Department of Biomedical Sciences and Human Oncology, Section of Internal Medicine and Clinical Oncology, University of Bari Medical School, Policlinico, Bari, Italy.

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Address for reprints: Vito Michele Lauta, M.D., Department of Biomedical Sciences and Human Oncology, Section of Internal Medicine and Clinical Oncology, University of Bari Medical School, Policlinico, Piazza Guilio Cesare 11, 70124 Bari, Italy; Fax: (011) 39-080-54-78-820; E-mail: dimoclin@cimedoc.uniba.it

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Because many studies have focused on growth factors in multiple myeloma, the study of the cytokine network appears to be useful for this purpose. Interleukin-6 (IL-6) and IL-2 with their soluble receptors (IL-3, IL-4, IL-10, and IL-11) have been examined. Plasma cells may produce IL-6 by an autocrine mechanism whereas a paracrine mechanism is believed to be involved in the production of IL-6 by bone marrow stromal cells through an interaction between adhesion molecules present on myeloma plasma cells and their respective receptors that are present on bone marrow stromal cells. In addition, control over production of IL-6 may be exerted by other ILs such as IL-1β and IL-10. Among target cells, the growth of normal and myeloma plasma cells is supported by IL-6, which also induces the differentiation of myeloma plasmablastic cells into mature plasma cells. This last action also is shared by IL-3, IL-4, and, most likely, IL-8. Evaluation of the serum level of IL-6, C reactive protein, soluble IL-6 receptor (sIL-6R), and soluble IL-2 receptor (sIL–2R), together with the activity exerted by IL-3 and IL-4 on some cellular subsets, may constitute an additional element in the differential diagnosis of borderline cases. However, the concomitant evaluation of all immunologic parameters could be more useful than the value of a single IL. Serum levels of IL-6, sIL-6R, sIL-2R, and the expression of membrane-bound IL-2 receptors, both on bone marrow plasma cells and on peripheral blood mononuclear cells, are correlated with disease activity and disease stage. In addition, IL-6 and sIL-6R serum levels are believed to be correlated with the duration of disease-free survival because a high serum level at the time of diagnosis is believed to be correlated with a short duration of survival. However, some laboratory parameters may express the same prognostic value as high β_2 microglobulin and lactate dehydrogenase (LDH) serum levels together with a high plasma cell labeling index are correlated with disease activity. Furthermore, if the evaluation is performed at the time of diagnosis, high values of these parameters are correlated with a short disease-free survival. A correlation between laboratory parameters and the serum level of several cytokines was demonstrated. Hence, the real advantage of the prognostic evaluation of cytokines is reserved for patients who do not exhibit uniform results with regard to β_2 microglobulin and LDH serum levels, or, better, for borderline cases. With regard to the differential diagnosis, all immunologic parameters should be evaluated concomitantly rather than separately to confer a real prognostic value to results. Furthermore, a particular relation was found between a high sIL-6R serum level and a poor response to chemotherapy, therefore suggesting the possibility of identifying in advance a subset of patients with a high risk of treatment failure, as has already been demonstrated in other hematologic malignancies.

Finally, the majority of studies indicate that interferons are used mainly in the immunotherapy for multiple myeloma, whereas many clinical trials should still be required for the evaluation of the effectiveness of anti-I-L6 antibodies or antiidiotypic vaccines in reference to the eligible patients for these particular therapies.
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Multiple myeloma is a plasma cell dyscrasia characterized by the slow proliferation of malignant plasma cells, primarily in the bone marrow. Many studies have focused on the network of cytokines that control growth, progression, and dissemination of the disease.[1] The network includes interleukin-1β (IL-1β), IL-2 and its soluble receptor, IL-3, IL-4, IL-6 and its soluble receptor, tumor necrosis factor-β (TNF-β), IL-10, and IL-11, even if each of them appears to be involved in a different manner in the biology of normal and malignant plasma cells.

IL-6 AND MULTIPLE MYELOMA

Generalities

IL-6 is a member of a cytokine superfamily that includes leukemia inhibitory factor, oncostatin M (OSM), ciliary neurotrophic factor, and IL-11. Family members share a common glycoprotein 130 receptor component involved in signal transduction across the cell membrane and, in the case of IL-6, there also is a specific receptor α-chain that is the glycoprotein 80 component (IL-6R). IL-6R is comprised of a highly glycosylated single polypeptide chain with a molecular weight of 80 kilodaltons. The intracytoplasmic portion of IL-6R has only 82 amino acid residues without any unique sequence for the signal transduction, suggesting the presence of another associated molecule responsible for the signal transduction that is the glycoprotein 130 receptor.

Sources of IL-6

With regard to the source of IL-6 in multiple myeloma, there is clear evidence to support both autocrine and paracrine mechanisms of IL-6 production. Paracrine mechanism is based on results coming from many experimental studies performed in the last few years. In some studies, some human myeloma cell lines (MM-S1, MM-A1, MM-Y1, and MM-C1) proliferated in response to IL-6, and immunologic staining with an anti-IL-6 receptor monoclonal antibody revealed the presence of receptors for IL-6 on cells from each cell line. Conversely, Northern blot analysis demonstrated that all tested cell lines did not express IL-6 messenger RNA, therefore suggesting that they are responsive to IL-6 but not by the autocrine mechanism. Furthermore, in other experimental studies, some myeloma-derived cell lines such as RPMI 8266 were shown to increase DNA synthesis when cultured with exogenous IL-6. The same results were obtained in other studies with regard to the dependence of the growth and survival of certain myeloma cell lines such as U-1958 and U-1996 on exogenous IL-6 activity. Therefore, the predominant source of IL-6 produced by a paracrine mechanism in multiple myeloma patients appears to be bone marrow stromal cells. Adhesion of myeloma plasma cells to bone marrow stromal cells and osteoblasts plays a critical role in IL-6 transcription and secretion by bone marrow stromal cells and osteoblasts.[2] Apart from the paracrine mechanism, constitutive autocrine IL-6 production by myeloma plasma cells has been shown to suggest that a possible autocrine mechanism of IL-6 may operate in the generation of human multiple myeloma. In some experimental studies a high concentration of IL-6 was detected in the supernatant fluid from a particular human myeloma cell line called U-266. Results coming from other experimental studies on seven myeloma cell lines (OCI-My 1–7) demonstrated that all cell lines expressed mRNA for the IL-6 receptor, whereas the expression of IL-6 mRNA was present in five of seven lines only. In addition, IL-6 protein was detected in the culture supernatant fluid of two cell lines only (OCI-My 3 and 2). The same heterogeneity of results was observed among three new myeloma cell lines (DP-6, KAS-6/1, and KP-6) that were established from patients with aggressive disease. Indeed, all three cell lines expressed IL-6 mRNA but only DP-6 and KP-6 cells were shown to be secreting biologically active IL-6. Taken together, all the elements that have been examined to date allow the identification of two main mechanisms of IL-6 production; the paracrine mechanism indicates that the great majority of myeloma cells do not produce IL-6 in vivo but that there might exist a minority of myeloma cells producing an autocrine IL-6.

Effects of IL-6 on Plasma Cells

IL-6 has been demonstrated to be involved in the proliferation of plasmablastic cells in bone marrow and in the differentiation of these cells into mature plasma cells. Apart from its involvement in the development of normal plasma cells, it now is clear that IL-6 is a potent myeloma cell growth factor involved not only in vitro but also in vivo. Several arguments may support this hypothesis; the degree of response of myeloma cells to IL-6 in vitro reportedly correlates with that of the proliferation of myeloma cells in vivo[3]; IL-6 serum levels are increased in patients with multiple myeloma and reflect disease severity; the inhibition of myeloma cell proliferation with antitumoral effects was observed in patients who had been given anti-IL-6 murine monoclonal antibodies.[4] In addition to the involvement of IL-6 in myeloma cell growth, a relation between this cytokine and C-reactive protein (CRP) has been demonstrated because high CRP serum levels have been observed in patients with active multiple myeloma. Therefore, CRP serum levels reflect IL-6 in vivo and they may be regarded as a powerful prognostic factor in patients with multiple myeloma.

The role exerted by IL-6 as a myeloma plasma cell growth factor also is supported by further clinical elements. IL-6 has been shown to be a potent bone resorbing factor and may be involved in the production of bone lesions in multiple myeloma patients. A case of a woman with both multiple myeloma and renal cell carcinoma was reported. Disease remission of multiple myeloma was observed after nephrectomy had been performed. A high serum level of IL-6 was found before nephrectomy whereas it decreased afterward. Because a high level of IL-6 in supernatant fluid from renal cell carcinoma cells cultured in vitro was detected, a stimulation of myeloma cell proliferation exerted by IL-6 produced by renal cell carcinoma cells was hypothesized.

With regard to the mechanism of action by which IL-6 may stimulate the growth of myeloma cell lines, the inhibition of apoptosis may be hypothesized because IL-6 has been shown to overcome the apoptosis of myeloma cells induced by glucocorticoids. Furthermore, the up-regulation of the cellular expression of *bcl*-XL protein may be considered to be an additional mechanism of action of IL-6 exerted at least on a specific myeloma cell line (i.e., IL-6-dependent B9 myeloma cells). In addition, the involvement of retinoblastoma protein (pRB) in the IL-6-mediated myeloma cell growth has been reported recently. In particular, IL-6 involved in the proliferation of myeloma cell lines was demonstrated to down-regulate dephosphorylated pRB and to shift the dephosphorylated pRB to its phosphorylated form, suggesting a possible mechanism of action of IL-6 to promote myeloma cell growth via phosphorylation of pRB.[5]

Interactions between IL-6 and Other Cytokines

As mentioned earlier, IL-1α, IL-2, IL-4, and IL-10 have been shown to stimulate the partial differentiation of B cells into normal plasmablastic cells, whereas IL-6 has not been proven to be a differentiation factor in normal B cells. Several cytokines may exert a combined action on different myeloma cell lines. Nonhomogenous expression on the cellular surface of specific receptors for different cytokines may explain the variegate pattern of cellular response. In particular, interferon-α (IFN-α) has been shown to stimulate the proliferation of some IL-6–dependent myeloma cell lines (such as XG-1, XG-2, XG-3, XG-4, and XG-5 cellular proliferations) by induction of the autocrine production of IL-6. Conversely, IFN-α has been shown to increase the duration of the plateau phase in the response of multiple myeloma patients to previous chemotherapy. Actually, these results are not necessarily in contradiction with the reported relation between IFN-α and IL-6 because myeloma cells from patients with inactive disease were shown not to be responsive to IL-6 in vitro. Compared with IFN-α, there are some lines of evidence that IFN-γ is a potent inhibitor of myeloma cell proliferation by the down-regulation of the expression of IL-6 receptor complex first chain. Furthermore, endogenous granulocyte-macrophage–colony-stimulating factor (GM-CSF), which is produced by the myeloma bone marrow microenvironment, was hypothesized to be involved in myeloma cell proliferation through the mediation of IL-6. Indeed, the stimulation of cellular proliferation was found to be abrogated by anti-IL-6 monoclonal antibodies. It has been demonstrated that IL-8 is produced by bone marrow stromal cells. Because IL-8 is chemotactic for neutrophils and lymphocytes, the hypothesis that IL-8 may be able to attract circulating malignant plasma cells precursors into a IL-6-rich bone marrow microenvironment must be verified. Furthermore, IL-3 has been demonstrated in vitro to have a proliferative activity on multiple myeloma plasma cells in synergy with IL-6. In addition, IL-3 levels in serum samples from patients with multiple myeloma were shown to be higher than those in normal subjects or patients with monoclonal gammopathies of uncertain significance. Moreover, some observations report that sera from patients with multiple myeloma may induce proliferation of a particular myeloma cell line (M-07). This proliferation is drastically reduced by the addition of anti-IL-3 neutralizing antibodies, thereby indicating the presence of IL-3. Taken together, these data confer to IL-3 the value of a prognostic and diagnostic parameter with regard to malignant and nonmalignant gammopathies. In reference to other cytokines, IL-10 has been clearly demonstrated to be the most potent B-cell differentiation factor. IL-10 stimulates the proliferation of freshly explanted myeloma cells in the IL-6-deprived cultures of tumor samples from patients with active multiple myeloma; therefore it can be regarded as an IL-6-unrelated growth factor for malignant plasmablastic cells. The mechanism of action by which IL-10 may exert its activity on myeloma cells is likely to be based on the production of a functional autocrine OSM loop in some myeloma cells (such as xG-1 and xG-2 cells) that express sensitivity to IL-10 activity. The relation between IL-6 and OSM has suggested the hypothesis of a possible correlation between this IL-6-related cytokine, OSM, and clinical and biochemical findings in patients with multiple myeloma.

Significance of IL-6 and the Network of Several Cytokines in Multiple Myeloma

Several reports have discussed a potential diagnostic role of the IL-6 serum level in different hematologic disorders such as multiple myeloma, monoclonal gammopathies of undetermined significance (MGUS),

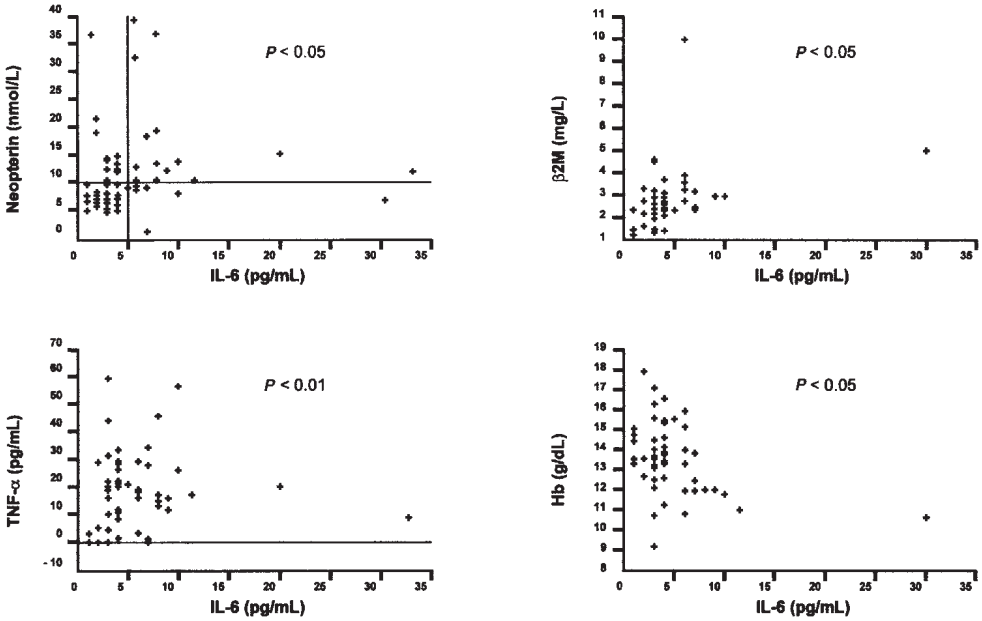

FIGURE 1. Correlations between serum levels of interleukin-6 (IL-6) and serum neopterin, β2-microglobulin (β2M), tumor necrosis factor-α (TNF-α), and hemoglobin (Hb) in patients with monoclonal gammopathies as defined by the Spearman rank correlation coefficient. Reprinted with permission from Nachbaur DM, Herold M, Maneschg A, Huber H. Serum levels of interleukin-6 in multiple myeloma and other hematological disorders: correlation with disease activity and other prognostic parameters. *Ann Hematol.* 1991;62:54–58. ©Springer-Verlag.

low-grade non-Hodgkin lymphomas (NHL), and myeloproliferative diseases. Results from many clinical studies indicate that the highest IL-6 serum level was found in patients with multiple myeloma (42%) as opposed to the level exhibited by those with the hematologic malignancies mentioned earlier (range, 1–15%). Conversely, higher serum IL-6 levels were found among patients with MGUS (16%) compared with patients with NHL or myeloproliferative diseases (range, 1–15%).[6] Results from other clinical trials have confirmed these data but they also have reported that high IL-6 serum levels were found in patients with acute non-lymphoblastic leukemia as well as in those with multiple myeloma.[7] These data confer an additional diagnostic value to IL-6 that may allow for the distinction of multiple myeloma from MGUS. Moreover, the high expression of IL-6 mRNA on cellular populations in MGUS patients could predict those patients whose disease eventually will progress to multiple myeloma.[8] In addition, in patients with multiple myeloma, the IL-6 serum level was found to be significantly higher in patients with disease at advanced stages (Stages II/III according to Durie and Salmon) or those with progressive disease compared with in patients with Stage I multiple myeloma or those whose disease was at the plateau phase. Furthermore, a strong correlation was found between the serum level of IL-6 and several parameters of disease activity because high levels of IL-6 were found to be correlated with high values of bone marrow plasmacytosis, serum lactate dehydrogenase (LDH), serum β_2 microglobulin, and serum neopterin (Fig. 1; Table 1). These findings proved that the serum level of IL-6 is a significant prognostic marker in multiple myeloma patients.[7] In accordance with this hypothesis, a relation between the duration of overall survival and the serum level of IL-6 was investigated. Results coming from some clinical studies demonstrated that survival times differed significantly between patients whose IL-6 levels were < 7 pg/mL at the time of diagnosis and those with IL-6 concentrations of ≥ 7pg/mL. The 50% survival rate in the former category was 53.7 months compared with only 2.7 months for the same percentage survival rate in the latter category of patients. These data obviously tend to confer to IL-6 a powerful prognostic value that could be confirmed by results regarding the kinetics of IL-6 levels during the course of the disease and under various forms of treatment. In contrast with the majority of study results, which demonstrate a real diagnostic and prognostic value for the serum level of IL-6 in patients with multiple myeloma, some other reports have not confirmed these data. A comparison of IL-6 plasma levels measured by enzyme-linked immunoadsorbent assay (ELISA) in normal volunteers, in patients with multiple myeloma, and in patients with benign monoclonal gammopathies has been shown to exhibit slight differences between the two groups of patients without any significant statistical significance. In addition, no correlation was found between IL-6 serum level and disease activity together with high levels of IL-6 associated with low tumor burden and low growth fraction. However, the controversial results that have been reported may express only an apparent contrast because they may depend on different methods that have been used to evaluate IL-6 activity. According to some authors there may be a difference between the bioassay of IL-6 activity and IL-6 plasma levels mea-

TABLE 1
Relations between Mean Serum IL-6 Levels and Parameters of Disease Activity in 55 Patients with Multiple Myeloma[a]

Parameters	No. of cases	Serum IL-6 levels, mean ± SD (pg/mL)	Median (pg/mL)	Range (pg/mL)	Statistical significance (Mann–Whitney *U* test)
Bone marrow plasmocytosis					
< 50%	28	335 ± 130	300	170–677	$P < 0.05$
≥ 50%	23	570 ± 263	579	218–947	
Serum LDH					
< 250 Ul/L	36	304 ± 110	274	153–571	$P < 0.05$
≥ 250 Ul/L	14	473 ± 240	397	171–947	
Serum β_2 microglobulin					
< 6 mg/L	36	326 ± 144	283	153–677	$P < 0.05$
≥ 6 mg/L	18	452 ± 212	396	246–947	
Hemoglobin level					
< 100 g/L	11	526 ± 248	441	209–947	$P < 0.01$
> 100 g/L	42	324 ± 132	298	153–747	
Serum calcemia					
< 105 mg/L	47	339 ± 168	299	153–947	$P < 0.05$
≥ 105 mg/L	8	536 ± 134	504	352–747	
Serum creatinine					
< 20 mg/L	42	347 ± 177	294	247–879	NS
≥ 20 mg/L	13	436 ± 166	405	153–947	
C reactive protein					
< 10 mg/L	35	335 ± 160	300	153–879	$P < 0.05$
≥ 10 mg/L	12	459 ± 204	460	207–947	
Serum albumin					
< 30 g/L	10	439 ± 225	423	170–879	NS
≥ 30 g/L	45	352 ± 164	311	153–947	
Serum thymidine kinase					
< 5 U/L	18	292 ± 114	240	170–571	NS
≥ 5 U/L	25	353 ± 192	305	153–947	

IL-6: interleukin-6; SD: standard deviation; LDH: lactate dehydrogenase; NS: not significant.

[a] Comparison of serum interleukin-6 levels between groups were made using the Mann–Whitney *u* test.

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sured by ELISA. In addition, some studies performed to evaluate the prognostic value of serum IL-6 levels in multiple myeloma patients demonstrated important daily variations in some patients[9] (Table 2), which may explain the apparent contrast mentioned earlier. Furthermore, a disturbance of the IL-2/IL-2 receptor system was demonstrated to have a powerful diagnostic and prognostic value in patients with monoclonal gammopathies. Experimental data from a study conducted by Vacca et al.[10] report that serum and urinary values of the soluble IL-2 receptor (sIL-2R) were significantly increased in multiple myeloma patients compared with normal controls. This increase also was related to active disease. B-cell preparations from peripheral blood mononuclear cells showed significantly increased proportions of IL-2R-positive cells in patients with multiple myeloma and MGUS; the highest proportions were detected in patients with active multiple myeloma compared with those with stable disease or MGUS. In addition, multiple myeloma patients had well defined IL-2R-positive plasma cell populations in their bone marrow, as opposed to patients with MGUS. Furthermore, the lowest serum IL-2 values were found in patients with active multiple myeloma. In conclusion, these findings indicate that a disturbance of the IL-2/IL-2R system can be used both in the diagnosis of multiple myeloma compared with MGUS and as an additional marker of active malignancy. As mentioned earlier, IL-3 may be indicated as a diagnostic factor regarding multiple myeloma and MGUS. Although this hypothesis is supported mainly by a higher IL-3 serum level found in patients with multiple myeloma compared with the level detected in patients with MGUS, it may be confirmed further by the effects of IL-3 combined with IL-6 on myeloma precursor cells from the peripheral blood of patients with multiple myeloma and those with MGUS. Indeed, in some experimental studies, nonadherent mononuclear cells (NMC) from the peripheral blood of these two different categories of patients were cultured in

TABLE 2
Variations in Interleukin-6 Levels in Patient Sera

Patient No.	Serum IL-6 levels (pg/mL)					
	Day 1		Day 2		Day 3	
	9 a.m.	5 p.m.	9 a.m.	5 p.m.	9 a.m.	5 p.m.
1	0	2293	0[a]	2209[a]	2329[a]	145[a]
2	2128[a]	ND	0[a]	ND	1491[a]	108[a]
3	2005	ND	52[a]	84	ND	ND
4	29	15	1820	0	2525	ND

IL-6: interleukin-6; ND: not done.

[a] Biologically controlled values.

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the presence of IL-3 and IL-6. After 10 days of culture, monoclonal-cytoplasmic-immunoglobulin (cIg)-positive plasma cells appeared from NMC in the majority of patients with multiple myeloma whereas these changes were not observed in patients with MGUS. The role exerted by IL-4 in patients with multiple myeloma appears to be controversial according to results obtained by several experimental studies. Conversely, interesting results have been obtained by Sawamura et al.[11] regarding the action exerted by IL-4 in combination with TNF-α on myeloma cell precursors. After incubation with IL-4 and TNF-α, monoclonal plasma cells appeared in peripheral blood mononuclear cells (PBMC) from patients with multiple myeloma but not from those with MGUS. This confers to this assay a diagnostic value to distinguish the early stage of multiple myeloma from MGUS. However, no correlation was found between the IL-4 serum level and disease status in multiple myeloma patients. Different results were obtained by experimental studies conducted to investigate the role exerted by IL-10 in patients with multiple myeloma. The IL-10 serum level was measured in patients with MGUS, in patients with early or advanced stage multiple myeloma, and in patients with fulminating disease (primary treatment failure or disease recurrence). The highest proportion of patients in whom IL-10 was detectable were those with early-stage disease compared with the lowest percentage of patients with advanced or fulminating disease. In addition, IL-10 was not detectable in the serum of individuals with MGUS. These data show that IL-10, like IL-6, might be involved in human multiple myeloma expressing a diagnostic value that may allow for the distinction between early-stage multiple myeloma and MGUS. However, unlike IL-6, a high detectable serum level of IL-10 is associated with a good prognosis. Nevertheless, these findings have not been confirmed because results from a recent clinical study conducted by Stasi et al.[12] do not appear to support an apparent involvement of IL-10 in multiple myeloma. Indeed, serum levels of IL-10 were measured in patients with multiple myeloma in various phases of the disease, in individuals with MGUS, and in healthy volunteers. In patients with multiple myeloma, the cytokine was detected with a comparable frequency in all pathologic stages and phases of the disease. Furthermore, IL-10 concentrations did not appear to differ significantly between controls and patients with plasma cell dyscrasia and between patients with MGUS and multiple myeloma.

Significance of sIL-6R in Multiple Myeloma

As mentioned earlier, a remarkable feature of the sIL-6R/IL-6 receptor complex is the agonist role exerted by the soluble form, which binds IL-6 as efficiently as the membrane-bound IL-6 receptor. The agonist role exerted by sIL-6R is comprised of the activation of the glycoprotein 130 transducer chain, which is involved in signal transduction across the cell membrane; it also may amplify circulating IL-6 in patients with multiple myeloma.[13] The ability of sIL-6R/IL-6 complex to activate the glycoprotein 130 transducer chain enables the soluble form to stimulate proliferation of myeloma cells that express either low or nondetectable surface IL-6 receptor. Many clinical and experimental studies have been conducted to investigate the clinical significance of sIL-6R in patients with several plasma cell dyscrasias. Identical results have been obtained by several authors[12–14] in reference to the diagnostic value because the sIL-6R serum level was shown to be higher in patients with malignant gammopathies compared with those with MGUS (Fig. 2). In reference to prognostic value in multiple myeloma patients, a correlation was found between disease activity and sIL-6R because its serum level was found to be higher in early or late active disease than in plateau-phase disease, in which it ranged within normal limits (Fig. 2). A strong correlation between sIL-6R and survival was confirmed by the results from the majority of studies published to date. Indeed, Kaplan–Meyer analysis demonstrated that elevated levels of sIL-6R were associated with shorter survival as opposed to the longer survival found among patients with low levels of sIL-6R at the time of diagnosis.[12,15]

Immunotherapy of Multiple Myeloma

Treatments closely connected to immunotherapy have been increasingly taking hold, including toxic IL-6, monoclonal antibodies directed against both IL-6 and IL-6 receptors, IL-6 receptor superantagonists, IL-2, and antiidiotypic vaccines. Toxic IL-6 has been prepared by the fusion of the IL-6 gene to genes of *Pseudomonas exotoxin* or diphteria toxin. The in

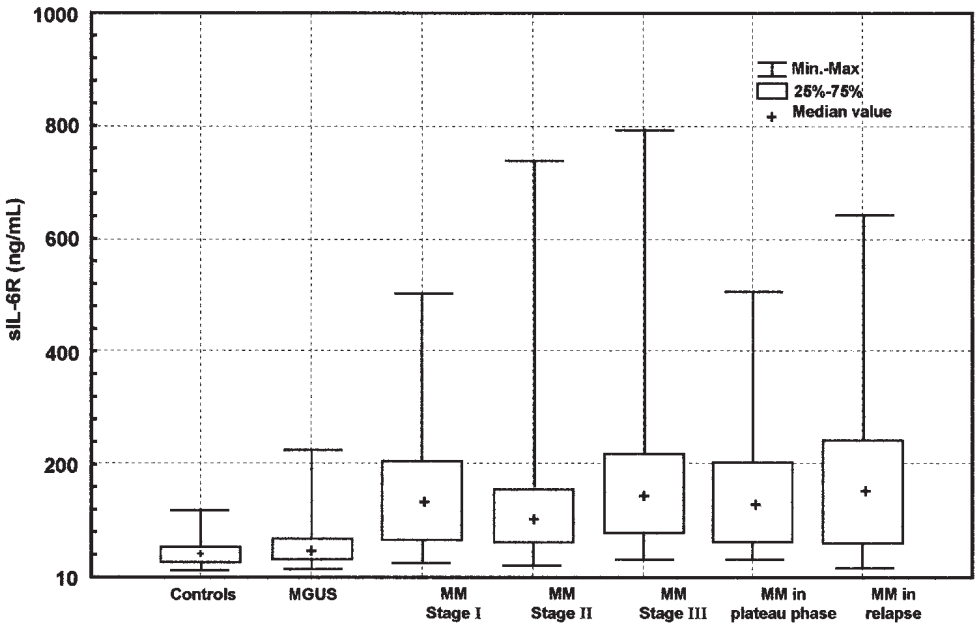

FIGURE 2. Soluble interleukin-6 receptor (sIL-6R) levels in patient groups and controls. MGUS: monoclonal gammopathy of undetermined significance; MM: multiple myeloma; Min: minimum; Max: maximum. Reprinted with permission from Stasi R, Brunetti M, Parma A, Di Giulio C, Terzoli E, Pagano A. The prognostic value of soluble interleukin-6 receptor in patients with multiple myeloma. *Cancer.* 1998;82:1860–1866. ©1998 American Cancer Society. Reprinted with permission of Wiley–Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

vivo use of toxic IL-6 as a single agent appears doubtful because numerous normal cells, particularly hepatocytes, do constitutively express the IL-6 receptor in vivo. The initial results of treatment with monoclonal anti-IL-6 antibodies has appeared promising. Some experimental studies showed that pretreatment of mice with anti-IL-6 monoclonal antibodies resulted in a significant reduction of mortality induced by inter peritoneal (i.p) implanted IL-6-dependent plasmacytoma cells. Furthermore, Klein et al. reported the treatment of a patient with a therapy-resistant plasma cell leukemia using a daily intravenous injection of murine anti-IL-6 monoclonal antibodies for 2 months. The patient's clinical status improved and was accompanied by a block of the myeloma cell proliferation in bone marrow and a reduction of serum levels of calcium, monoclonal component, and CRP. The same group has successively presented data regarding multiple myeloma patients treated with murine anti-IL-6 antibodies administered at doses of 20 mg/day for 6 days. The preeminent result of this treatment was a dramatic blockage of myeloma cell proliferation in vivo with mild thrombocytopenia as the only side effect. In accordance with these observations, in other clinical studies a new methodology to estimate daily IL-6 production in patients with multiple myeloma or renal cell carcinoma who received anti-IL6 monoclonal antibodies. The measurement of whole body IL-6 production was found to be predictive of the efficacy of anti-IL-6 therapy. Results demonstrated that in responsive patients, a low whole-body IL-6 production was found (< 18 μg/day) as opposed to the higher value observed in nonresponsive patients (180 μg/day). Subsequently, the efficacy of the administration of anti-IL-6 monoclonal antibodies to multiple myeloma patients was investigated by Van Zaanen et al. in a Phase I clinical study.[16] The patients enrolled in this trial had developed disease recurrence after a second-line treatment such as the vincristine, doxorubicin, and dexamethasone (VAD) regimen or high-dose melphalan with or without autologous stem cell transplantation. An escalating dose of chimeric anti-IL-6 monoclonal antibodies (cMab) was used (5–40 mg/die from Day 0–14 and Day 28–42). Results showed low toxicity, low immunogenicity, and long half-life of monoclonal antibodies that were present in the form of biologically inactive IL-6–anti-IL-6 cMab complexes. Although CRP serum levels were decreased, indicating a neutralization of IL-6 activity, no clinical response was observed in association with stable values of the monoclonal component (Fig. 3). The dissociation between the neutralization of IL-6 activity and the lack of responsiveness has been explained by the different biologic properties exhibited by plasma cells in patients with multiple myeloma. Indeed, immature plasma cells are considered to be proliferating whereas mature plasma cells display a low proliferative activity with higher secretion of M-protein. The neutralization of IL-6 activity, induced by specific monoclonal antibodies, inhibits the proliferation of the highly proliferative clone whereas the production of M-protein by mature plasma cells appears to be less affected. The authors conclude that a Phase II clinical study could have yielded better results with regard to the administration of different doses of a cMab. More recently, the efficacy of a combined therapy including anti-IL-6 monoclonal antibodies in association with hormonal therapy and chemotherapy

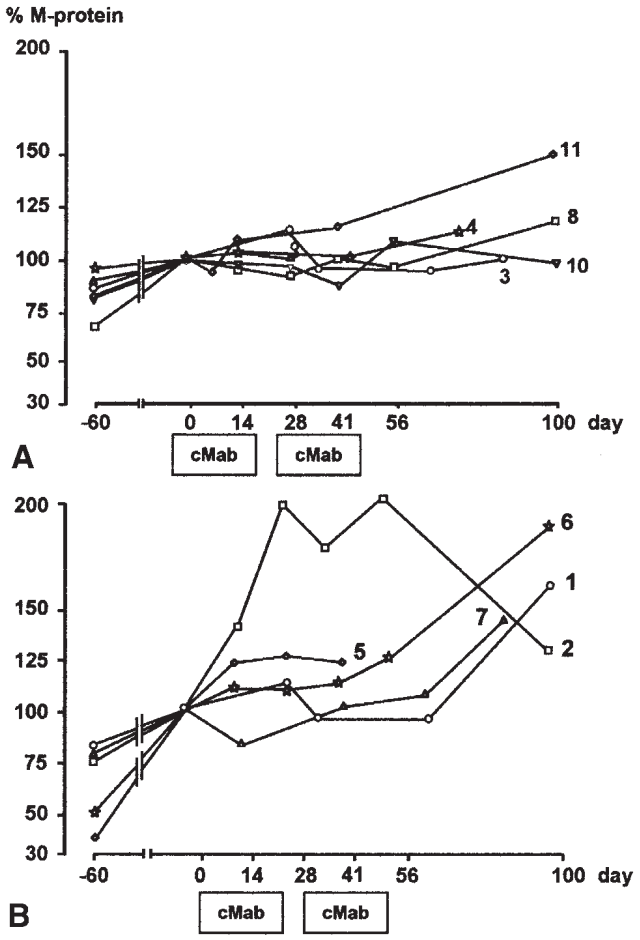

FIGURE 3. Percentage of M-protein levels before, during, and after antiinterleukin-6 (IL-6) treatment in relation to Day 0 (Day 0 = 100%). Anti-IL-6 chimaeric monoclonal antibodies (cMab) were given in 2 cycles of 14 days (2 boxes below abscissa). (A) Patients 3, 4, 8, 9, 10, and 11. (B) Patients 1, 2, 5, 6, and 7. At Day 60, Patient 2 received intravenous melphalan (70 mg/m^2). M-protein: monoclonal protein; anti-IL-6 cMab: chimaeric antiinterleukin-6 monoclonal antibodies. Reprinted with permission from Van Zaanen HCT, Lokhorst HM, Aarden LA, et al. Chimaeric anti-interleukin-6 monoclonal antibodies in the treatment of advanced multiple myeloma: a phase I dose-escalating study. *Br J Haematol.* 1998;102:783–790. ©1998 Blackwell Science, Ltd.

was investigated by Moreau et al.[17] in the treatment of patients with advanced multiple myeloma. A series of 16 patients (14 patients whose disease recurred after previous chemotherapy) was treated with a combination of BE-8 (an anti-IL-6 murine monoclonal antibody) and dexamethasone followed by high-dose melphalan and autologous stem cell transplantation. In all patients, a strong inhibition of IL-6 activity was observed that also was correlated with a high complete response rate. In conclusion, the analysis of the previously mentioned results indicates that anti-IL-6 monoclonal antibodies have been administered mainly to patients with end-stage disease. In addition, low IL-6 producers appear to be the most responsive patients. With regard to IL-6 receptor antagonists, it has been shown that they were produced in the form of two variants: the site 2 antagonist and the site 2+3 antagonist.[18] Indeed, IL-6 has been shown to possess three topologically distinct receptor binding sites: site 1 for binding to the subunit-specific glycoprotein 80 component of the IL-6 receptor and sites 2 and 3 for the interaction with two subunits of the signalling chain glycoprotein 130. IL-6 receptor antagonists are particular IL-6 variants that carry substitutions that abolish interaction with glycoprotein 130 at either site 2 alone (site 2 antagonist) or at both sites 2 and 3 (site 2 and 3 antagonist). In addition, substitutions have been introduced in site 1 that were reported to lead to variable increases in binding for glycoprotein 80 IL-6 receptor up to 70-fold. IL-6 receptor superantagonists were shown to inhibit IL-6 activity with an efficacy that was proportional to the increase in receptor binding on human myeloma cell lines. These findings suggested that IL-6 receptor superantagonists could constitute IL-6 blocking agents in vivo, a finding that is particularly relevant for the treatment of multiple myeloma. In addition to the use of IL-6 receptor antagonists in the treatment of multiple myeloma, an interesting topic in the immunotherapy for this hematologic disorder may be the use of antiidiotypic vaccines. The variable regions of immunoglobulin heavy and light chains combine to form a unique antigen-recognition site of antibodies. In addition, they contain determinants that can themselves be recognized as antigens or idiotypes. In a study conducted by Bergenbrandt et al.[19] some patients with multiple myeloma were repeatedly immunized with the autologous monoclonal immunoglobulin G. The induction of idiotype-specific immunity was demonstrated by the amplification of the antiidiotypic T-cell response as well as by the increase in the number of B-cells secreting antiidiotypic antibodies. In another clinical study conducted by Kwak et al.[20], an antiidiotypic-specific response was reportedly obtained in a healthy sibling donor who had been immunized with the plasma of the recipient. All the more interesting is the fact that the myeloma idiotype-specific immunity could be transferred successfully to the recipient after the bone marrow transplantation. The results obtained by the idiotypic vaccination with regard to the induction of an idiotype immunization prompted some investigators to consider this therapy to be an adjuvant treatment for multiple myeloma patients in complete response after conventional chemotherapy.[20,21] Remarkable results were obtained by Massaia et al.[22] using the administration of autologous idiotype-specific proteins conjugated to keyhole limpet hemocyanin (KLH) associated with GM-CSF in pa-

TABLE 3
Effect of ID Vaccines on the Tumor Mass and Clinical Outcome

UPN	Immuno adjuvant	Serum M protein (mg/dL)		Serum k/λ ratio		%BM plasma cells		Immunofixation		FFDP (mos)	OS (mos)
		Pre	Post	Pre	Post	Pre	Post	Pre	Post		
14	GM-CSF	196	299	1,2	1,6	1	1	Pos	Pos	17	32+
240	GM-CSF	1359	1528	1,8	1,8	1	1	Pos	Pos	31+	31+
453	IL-2	1734	1755	0,8	0,6	1	3	Pos	Pos	36	39+
485	IL-2	1207	1189	2,4	2	ND	ND	Neg	Neg	14	33
510	GM-CSF	167	311	1,4	1,6	1	1	Pos	Pos	32+	32+
522	GM-CSF	1285	1388	0,6	0,4	2	5	Pos	Pos	19	36+
535	GM-CSF	63	122	1,6	1,4	1	1	Pos	Pos	15	19+
637[a]	GM-CSF	148	1258	1,8	0,7	1	2	Pos	Pos	4[a]	20
734	GM-CSF	1563	1486	14,9	10,3	5	5	ND	ND	17+	17+
743	GM-CSF	0[b]	0,5[b]	1,2	1,4	1	10	Neg	Neg	9	20+
749	GM-CSF	627	738	2,1	2	1	1	Pos	Pos	20+	20+
847	GM-CSF	1632	1412	4,4	3,3	2	2	Pos	Pos	11+	11+

ID: idotypic; UPN: unique patient number; Serum M protein: serum monoclonal protein; BM: bone marrow; FFDP: freedom from disease progression (calculated from the first immunization to the date of first treatment after vaccination or last follow-up); OS: overall survival (calculated from the first immunization to the date of death or last follow-up): Pre: prevaccine value; Post: postvaccine value; GM-CSF: granulocyte-macrophage–colony-stimulating factor; Pos: positive; IL-6: interleukin-6; ND: not done; Neg: negative.

[a] Treatment was not completed by this patient because of disease progression.

[b] Light chain disease (values are expressed as g/24 hours).

tients with multiple myeloma. A generation of tumor-specific immune responses were obtained. In particular, anti-KLH soluble and cellular immune responses were present in all patients compared with a poor antiidiotypic response. No disease progression was found during the follow-up period. The duration of progression-free survival and the overall survival ranged from 4 to 36 months and from 11 to 39 months, respectively (Table 3). An interesting method by which the idiotype-specific immune therapy may be performed might rely on the use of a potent and particular system of antigen presentation. It is comprised of idiotypic protein-pulsed dendritic cells because they are considered to be the key to activation of the immune system.[23] Indeed, peripheral blood-adherent mononuclear cells have been pulsed with autologous idiotype conjugated with KLH and then reinfused in the same patients. The development of both a soluble and cellular immune response to KLH was obtained mainly together with a less effective antiidiotypic response.[24–26] In a clinical study conducted by Reichardt et al., some multiple myeloma patients were previously subjected to high-dose chemotherapy followed by peripheral blood stem cell transplantation. Subsequently, they were treated with idiotype-pulsed autologous dendritic cells followed by idiotype/KLH. Results showed an effective antiidiotypic T-cell response together with improved clinical features comprised of a minimum follow-up of 16 months for the majority of patients in complete response.[27] Nevertheless, the antiidiotypic immune responses may be inhibited by major histocompatibility complex (MCH) monoclonal antibodies. Indeed, the immunization of myeloma patients by autologous M-component may evoke the production of MCH monoclonal antibodies directed against CD4-specific and CD8-specific T-cells inducing a restriction of T-cell responses.[28] The use of IL-2 in the treatment of patients with multiple myeloma is based on the modulating function exerted by some cells belonging to the immune system on tumor cell growth. In some clinical studies, IL-2 was used to treat some patients with advanced stage multiple myeloma who had failed standard chemotherapy. Results showed that the diminished pretreatment $CD4^+/CD8^+$ ratio was normalized together with an enhancement of natural killer (NK) and lymphokine-activated killer cell activity. Equivalent clinical features were comprised of an objective reduction in the tumor mass with long-lasting stable disease after disease progression before the initiation of IL-2 treatment. The termination of tumor progression rather than tumor regression exhibited by the majority of responding patients suggested that IL-2 therapy could be used for the maintenance of chemotherapy-induced disease remission. Among several cytokines that are provided with antineoplastic activity, interferons (IFNs) have been shown to be effective in the treatment of hematologic malignancies. In particular, recombinant IFN-α possesses this activity and the mechanism of action is based on its ability to exert a direct antipro-

liferative activity, an enhancement of the expression of tumor-associated antigens on the neoplastic cell surface and the stimulation of the cytotoxic activity of killer or NK cells. The most impressive responses were obtained in the treatment of hairy cell leukemia and chronic myelogenous leukemia.[29] With regard to multiple myeloma, both in vivo and in vitro studies have been performed to investigate the effectiveness of IFN in the treatment of this hematologic disorder. In vitro studies have demonstrated that the production of monoclonal immunoglobulin by myeloma plasma cells is reduced by IFN-α together with the inhibition of myeloma cell line development. However, results observed in the majority of in vitro studies are not identical because IFN-α also has been shown to suppress dexamethasone-induced apoptosis. The suppression of apoptosis was found to be concurrent with the induction of both AP-1 and STAT binding activity.[30] In any case, the results obtained by in vivo studies appear to be more homogeneous in reference to the effectiveness of IFN in the treatment of patients with multiple myeloma. Clinical trials performed to evaluate the efficacy of IFN in the treatment of human multiple myeloma have demonstrated that the treatment of previously untreated patients with IFN is not useful because the response rate is lower than that obtained with chemotherapy.[31] There is no homogeneity in the results of combined chemotherapy plus IFN given as first induction treatment because the benefit induced by the addition of IFN to chemotherapy has not been confirmed in all randomized studies when it is compared with chemotherapy alone.[31] In addition, homogeneous results have not been obtained using IFN alone or in combination with chemotherapy as second induction treatment in patients with recurrent disease. Indeed, this combined therapy has not always been shown to induce a real advantage if it is compared with chemotherapy alone.[31] However, IFN appears to be effective as maintenance therapy in patients who have achieved a response with previous chemotherapy,[32–35] or after allogeneic[36] and autologous bone marrow transplantation.[37] In addition, patients treated with IFN as maintenance therapy were found to have a better duration of disease-free survival compared with those who did not receive any treatment (Figs. 4, 5). Results from the PETHEMA study[38] indicate a longer disease-free duration for patients treated with maintenance therapy compared with patients who did not receive this treatment (Fig. 6). Instead, no difference was found with regard to the survival duration for patients who received IFN compared with those allocated to the no treatment arm (Fig. 7). Features at the time of disease recurrence were similar in both groups as was the survival from the time of disease recurrence. In addition, the effectiveness of maintenance therapy with IFN-α and prednisone versus that associated with the use of IFN alone was evaluated by Salmon et al.[39] After 48 months, a better percentile of patients (30%) with progression-free survival was observed among those treated with IFN and prednisone compared with that observed among patients treated with IFN alone (10%). Furthermore, the efficacy of two different dosages of IFN-α-2b given as maintenance therapy to multiple myeloma patients was evaluated by Offidani et al.[40] Patients with multiple myeloma in the plateau phase were divided into 2 groups to receive IFN therapy at a dose of 3 MU 3 times a week (Group A) versus 3 MU/die (Group B). Results demonstrated a longer median duration of progression-free survival (38 months) in Group B patients compared with Group A patients (12 months). In addition, patients who were treated with an IFN dose of $>$ 30 MU/month experienced a longer

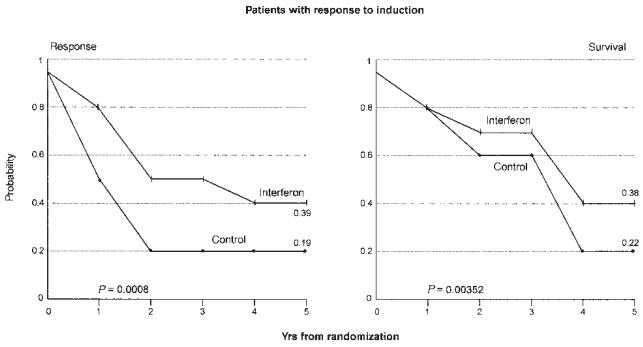

FIGURE 4. Kaplan–Meier curves for response and survival after randomization of patients to the interferon group or the control group.Reprinted with permission from Mandelli F, Avvisati G, Amadori S, et al. Maintenance treatment with recombinant interferon alpha-2b in patients with multiple myeloma responding to conventional induction chemotherapy. *N Engl J Med.* 1990;322: 1430–1434. ©1990 Massachusetts Medical Society. All rights reserved.

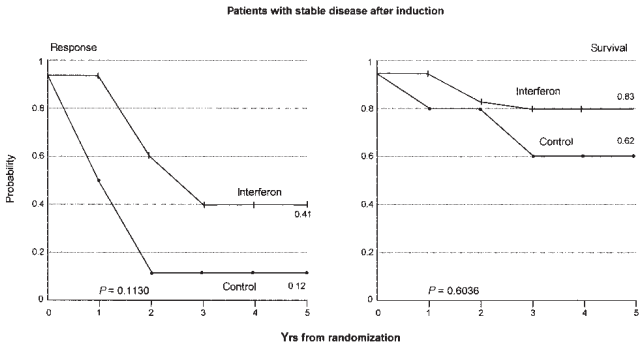

FIGURE 5. Kaplan–Meier curves for response and survival after randomization of patients to the interferon group or the control group. Reprinted with permission from Mandelli F, Avvisati G, Amadori S, et al, Maintenance treatment with recombinant interferon alpha-2b in patients with multiple myeloma responding to conventional induction chemotherapy. *N Engl J Med.* 1990;322: 1430–1434. ©1990 Massachusetts Medical Society. All rights reserved.

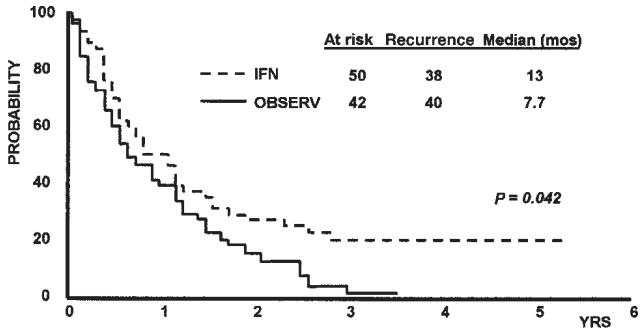

FIGURE 6. Duration of response from the time of randomization to the date of disease recurrence. IFN: interferon; Observ: observed group. Reprinted with permission from Bladé J, San Miguel JF, Escudero ML, et al. Maintenance treatment with interferon alpha-2b in multiple myeloma: a prospective randomized study from PETHEMA (Program of the Study and Treatment of Hematological Malignancies, Spanish Society of Hematology). *Leukemia.* 1998; 12:1144–1148.

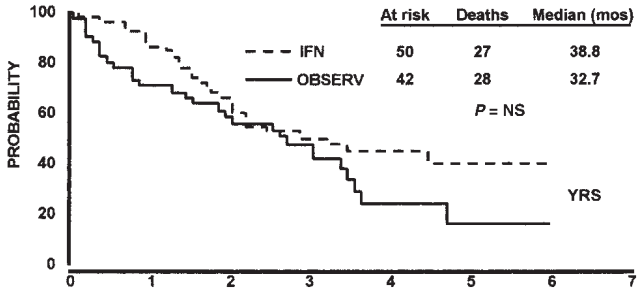

FIGURE 7. Survival from the time of randomization. IFN: interferon; Observ: observed group. Reprinted with permission from Bladé J, San Miguel JF, Escudero ML, et al. Maintenance treatment with interferon alpha-2b in multiple myeloma: a prospective randomized study from PETHEMA (Program of the Study and Treatment of Hematological Malignancies, Spanish Society of Hematology). *Leukemia.* 1998;12:1144–1148.

progression-free survival than the other patients. The conclusion is that the dose of IFN most likely is critical for obtaining a longer progression-free survival although the quality of life should be taken into account carefully. In addition to results obtained by Cunningham et al.[37] in reference to the effectiveness of maintenance therapy with IFN in patients with disease in a plateau phase after autologous bone marrow transplantation, further confirmation has been reported by the European Group for Blood and Marrow Transplantation (EBMT). In a retrospective registry study, a better median duration of overall survival and progression-free survival was observed among IFN-treated patients (78 months and 28 months, respectively) compared with that exhibited by patients who had not received maintenance therapy (47 months and 20 months, respectively). Furthermore, the difference in overall survival and progression-free survival was shown to be more evident among patients who achieved a partial response after autologous bone marrow transplantation than among those who achieved a complete response.[41] Side effects related to IFN therapy usually are reversible and are comprised mainly of flu-like symptoms and occasionally anemia, leukocytopenia, thrombocytopenia, an increase in the transaminase or creatinine serum level, weight loss, dermatitis, itching, and involvement of the peripheral nervous system. A particular note must be made concerning the effects of IFN-α on serum β_2 microglobulin. β_2 microglobulin forms the small invariable light chain subunit of the Class 1 histocompatibility leukocyte antigen (Class 1 HLA) antigens on the cell membrane of all nucleated cells. During the continuous turnover of the HLA molecules, β_2 microglobulin is shed from the cell membrane into the blood. Serum β_2 microglobulin has been reported to have significant prognostic value in lymphatic malignancies. However, because IFN-α has the ability to enhance the expression of Class 1 and 2 HLA, it may cause an increase in the formation and release of β_2 microglobulin. Therefore, the use of IFN-α abolishes the prognostic value of β_2 microglobulin, although its serum level is regarded as a marker of tumor burden. To our knowledge, IFN-β has not been widely employed in the treatment of multiple myeloma. However, some reports indicate a possible effectiveness in the treatment of patients with refractory or recurrent disease after first-line treatment. In some clinical studies an overall response rate of 75% was observed among patients with recurrent or refractory disease. Stable disease and a minor response were included in the range of responses. With regard to IFN-γ, it has been shown to be able to inhibit IL-6 activity through down-regulation of IL-6 receptors. Furthermore, a possible block of the IL-6 signal transduction pathway has been suggested because of the interaction with cytoplasmic proteins such as p91. These in vitro data encouraged pilot studies to evaluate the in vivo antitumor effects of IFN-γ. Preliminary results are controversial and the real role of this cytokine has not yet been established because of the lack of widespread and controlled studies.

Conclusions and Future Prospects

Analysis of the biologic properties of several cytokines that have been described to date allows us to draw some conclusions. Plasma cells may produce IL-6 by an autocrine mechanism, whereas a paracrine mechanism is involved in IL-6 production by bone marrow stromal cells. Among target cells, the growth of normal and myeloma plasma cells is supported by IL-6, which also induces the differentiation of myeloma plasmablastic cells into mature plasma cells. With regard to

the diagnostic value, the value of the IL-6 serum level, CRP, sIL-6R, and sIL-2R have been found to be higher in patients with multiple myeloma compared with those with MGUS. In addition, the value of IL-2 has been found to be lower in multiple myeloma compared with MGUS. Moreover, some elements of cellular biology may play a role in the differential diagnosis between MGUS and multiple myeloma because IL-3 and IL-4 have been shown to stimulate in vitro the differentiation of myeloma cell precursors into mature plasma cells at a higher level in patients with multiple myeloma compared with those with MGUS. The prognostic value of ILs may be considered to be their most useful property. All the ILs that have been examined to date have exhibited this value in different ways. Indeed, the serum level of IL-6, sIL-6R, and sIL-2R and the expression of IL-2 membrane receptors both on bone marrow plasma cells and on PBMCs are correlated with disease activity and the stage of disease. In addition, the serum levels of IL-6 and sIL-6R are reportedly correlated with the duration of disease-free survival because a high serum level at the time of diagnosis is connected to a short duration of survival. However, this prognostic value also is expressed by already known laboratory parameters. The real advantage of the prognostic evaluation of cytokines is limited to patients who do not exhibit uniform results for β_2 microglobulin, LDH serum levels. and the plasma cell labeling index value. Consequently, the prognostic evaluation of ILs appears to be useful mainly for borderline cases. Furthermore, a high sIL-6R serum level has been shown to be correlated with a poor response to chemotherapy. Therefore, apart from the predictive value expressed by several cytokines with regard to the duration of progression-free survival, the evaluation of prognostic factors at the time of diagnosis may allow us to identify in advance a group of patients who will not benefit from first-line treatment. They may be regarded as patients with a high risk of treatment failure as already demonstrated both in multiple myeloma patients by β_2 microglobulin together with plasma cell labeling index values and in patients with other hematologic malignancies by cytokine serum levels. Finally, with regard to the possible use of some ILs in the treatment of multiple myeloma, the majority of studies published to date have indicated that IFNs are mainly employed in the immunotherapy of this hematologic disorder. Instead, many clinical trials still are required for the evaluation of the effectiveness of anti-IL-6 antibodies and antiidiotypic vaccines in the patients who are eligible for these particular therapies.

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