Advances in Brief

Myeloma Cells Release Soluble Interleukin-6Rα in Relation to Disease Progression by Two Distinct Mechanisms: Alternative Splicing and Proteolytic Cleavage[1]

Wilfrid Thabard,[2] Sophie Barillé,[2] Madeleine Collette, Jean-Luc Harousseau, Marie-Josée Rapp, Régis Bataille, and Martine Amiot[3]

Institut National de la Santé et de la Recherche Médicale Unité 463 [W. T., S. B., M. C., R. B., M. A.], Institut de Biologie, and Département d'Hématologie Clinique [J-L. H., M-J. R.], 44093 Nantes cedex 01, France

Abstract

Multiple myeloma (MM) is a plasma-cell malignancy characterized by the accumulation of malignant plasma cells within the bone marrow. Interleukin (IL)-6 is an essential survival and growth factor for myeloma cells that exerts its activity through a cell surface receptor composed of an 80-kDa ligand binding molecule (IL-6Rα) and a 130-kDa signal-transducing molecule. Of major interest, the soluble form of the IL-6Rα (sIL-6Rα) is an agonistic molecule able to potentiate IL-6 activity and a strong prognostic factor in MM. In the present study, we demonstrate that purified myeloma cells from all of the patients with MM and human myeloma cell lines release sIL-6Rα. The level of sIL-6Rα release correlates with disease activity and is clearly up-regulated during tumoral expansion *in vivo* and immortalization *in vitro*. Of note, this sIL-6Rα release is strongly reduced (50%) by a hydroxamate-based metalloproteinase inhibitor underlying the importance of shedding in the production of sIL-6Rα by myeloma cells. Using specific IL-6Rα primers flanking the transmembrane domain, we demonstrate by PCR the presence of two IL-6R mRNAs corresponding to the membrane IL-6Rα and to the sIL-6Rα generated through alternative splicing in myeloma cells. In conclusion, we show that: (*a*) native myeloma cells and human myeloma cell lines release sIL-6Rα by two distinct mechanisms: alternative splicing and proteolytic cleavage of the membrane IL-6Rα; and (*b*) the release of the sIL-6Rα, which is an agonist of IL-6, correlates with disease progression, explaining in part its strong prognostic value *in vivo*.

Introduction

MM[4] is a plasma-cell malignancy characterized by the accumulation of malignant plasma cells within the bone marrow. IL-6 is an essential survival and growth factor for myeloma cells (1, 2). It exerts its activity through a cell surface receptor composed of an 80-kDa ligand binding molecule (IL-6Rα, CD126) and a 130-kDa signal-transducing molecule (gp130, CD130; Ref. 3). The soluble form of the IL-6Rα (*i.e.,* sgp80, sIL-6Rα) is an agonistic molecule that has been shown to potentiate IL-6 activity both *in vitro* and *in vivo* (4–6). In MM, two important observations have been made concerning the sIL-6Rα. First, elevated serum sIL-6Rα levels have been found in MM patients in correlation with a poor prognosis (7). Moreover it has been shown that elevated serum sIL-6Rα levels are higher in MM than in monoclonal gammopathy of undetermined significance (MGUS), allowing to discriminate MM patients from MGUS patients (8). Second, sIL-6Rα increases the proliferative response of myeloma cells to IL-6 and again behaves as a potent agonist of IL-6 (9, 10).

The sIL-6Rα seems to be generated through two nonexclusive mechanisms: (*a*) a proteolytic cleavage of the membrane IL-6Rα by a metalloproteinase (11, 12), and (*b*) the production of a sIL-6Rα form lacking the transmembrane domain (13, 14). In HMCLs, it has been shown that a metalloproteinase inhibitor significantly reduced the release of sIL-6Rα without abolishing it (12). Moreover, an mRNA encoding a soluble form of the IL-6Rα has been identified in the U266 HMCL (14). Therefore, we have investigated the capacity of native myeloma cells to release sIL-6Rα and the mechanism involved in the generation of sIL-6Rα. We demonstrate that myeloma cells themselves release sIL-6Rα by two distinct mechanisms, alternative splicing and proteolytic cleavage of the membrane IL-6Rα, and we determined the contribution of both mechanisms. Furthermore, we show that this release correlates with disease progression, explaining in part the strong prognostic value of serum sIL-6Rα in patients with MM.

Materials and Methods

mAbs and Reagents. The hydroxamate-based metalloproteinase inhibitors BB-3103 and BB-94 were kindly provided

Received 3/5/99; revised 7/12/99; accepted 7/24/99.

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

[1] Supported by Grant 9659 from Association pour la Recherche sur le Cancer and Programme Hospitalier de Recherche Clinique Grant BRD/97/8-K.

[2] Both authors contributed equally to this work.

[3] To whom requests for reprints should be addressed, at U463, Institut de Biologie, 9, quai Moncousu 44093 Nantes cedex 01, France. Phone: 33-2-40-08-47-66; Fax: 33-2-40-08-47-78; E-mail: mamiot@nantes.inserm.fr.

[4] The abbreviations used are: MM, multiple myeloma; IL, interleukin; HMCL, human myeloma cell line; PMA, phorbol 12-myristate 13-acetate.

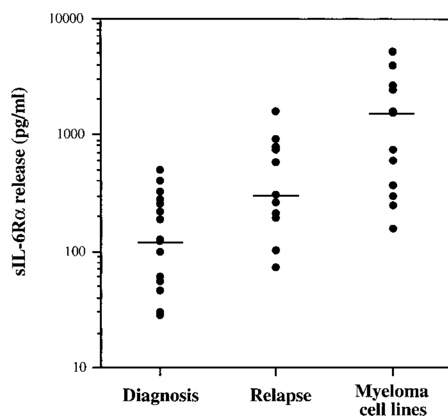

Fig. 1 Changes in sIL-6Rα release on myeloma cells according to disease activity in patients with MM; comparison with HMCLs.

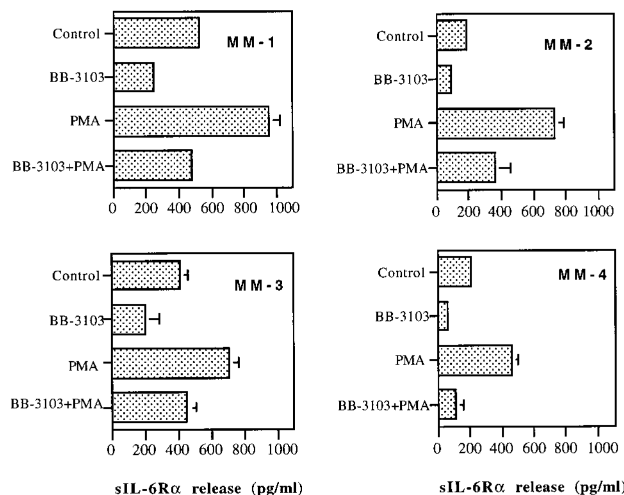

Fig. 2 Inhibition induced by BB-3103 on constitutive and PMA-stimulated sIL-6Rα release from native myeloma cells. Cells were plated at the density of 10^6/ml in the presence of the different reagents (25 μM PMA and/or 25 μM BB-3103), as indicated. After a 24-h incubation period, supernatants were collected and used for sIL-6Rα determination.

by British Biotech Pharmaceuticals Limited (Oxford, United Kingdom; Ref. 15). PMA was from Sigma Chemical Co. (St. Louis, MO). B-B4, an anti-CD138 mAb was a gift from Dr. J. Wijdenes (Diaclone Research, Besançon, France). Purified B-B4 was biotinylated as described previously (16).

Patients. Thirty-five MM patients have been included in the current study. The diagnostic criteria for MM were those of the Southwest Oncology Group (17). Nineteen MM patients were previously untreated (diagnosis), and 16 patients had progressive disease (relapse).

HMCLs. LP1, OPM-2, and NCI-H929 were purchased from DSM (Scheiwg, Germany). JJN3 and AMO-1 were kindly provided by Drs. B. Van Camp and J. Minowada, respectively. U266 and RPMI-8226 were obtained from the American Type Culture Collection. XG-1, XG-2, XG-6, SBN-1, and MDN were established previously in the laboratory. BCN was recently established in the laboratory from a patient with a secondary plasma cell leukemia. BCN displays a specific phenotype of myeloma cell line (*i.e.,* $CD138^+$, $CD38^+$, $CD19^-$, and $CD28^+$). The XG-, SBN-1, MDN, and BCN cell lines were dependent on IL-6 for their growth and were cultured in the presence of 3 ng/ml recombinant IL-6.

Purification of Myeloma Cells and Preparation of Conditioned Media. Mononuclear cells were isolated by Ficoll-Hypaque density centrifugation from bone marrow aspirates or peripheral blood from patients with MM or plasma cell leukemia. The percentage of plasma cells was then determined by morphology (May-Grunwald-Giemsa staining) and checked by flow cytometry using two-color staining with anti-CD38 and anti-B-B4 mAbs, as described (16). Only samples with a plasmocytosis superior to 8% were used for the subsequent purification. The purification of myeloma cells was performed using a MACS separator (Miltenyi Biotec, Germany). Cells were incubated with MACS microbeads coupled to CD138 before proceeding to magnetic separation on MS^+ separation column (Miltenyi Biotec). The purity of myeloma cells was evaluated by standard morphology (May-Grunwald-Giemsa stained cytospins), and only cell populations with a purity above 99% were used for the preparation of conditioned media. $B\text{-}B4^+$ myeloma cells were seeded in 96-well plates at the concentration of 10^6 cells/ml, and media were harvested after 24 h. The cultures were done in RPMI 1640 with 2% FCS. In all cases, the labeling index of myeloma cells was $<3\%$, thus, the number of myeloma cells after 24 h of culture was not significantly increased.

Determination of Soluble gp80 Production. The concentration of soluble gp80 in cell supernatants was determined using a sandwich ELISA (Pelikine Compact, Tebu, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, the Netherlands). The sensitivity of the test was 10 pg/ml. Assays were performed according to the manufacturer's recommendations.

RNA isolation and Reverse Transcription-PCR Amplification. Total cellular RNAs were prepared from 2.10^6 purified myeloma cells using Trizol (Life Technologies, Inc.). All RNAs were reverse transcribed with 400 units of Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.), according to the manufacturer's protocol. PCR was performed in a thermal-cycler (PCR Express; Hybaid) for 35 cycles of denaturation at 94°C for 1 min, followed by annealing at 62°C for 1.5 min and extension at 72°C for 1 min. Oligonucleotides used for PCR of IL-6Rα were as described by Horiuchi *et al.* (13): IL-6R-A (Thr 313) 5′-ACGCCTTGGACAGAATCCAG-3′ and IL-6R-B (Pro 456) 5′-TGGCTCGAGGTATTGTCAGA-3′.

Results

Myeloma Cells from All Patients with MM and HMCL Release sIL-6Rα, and Levels of sIL-6Rα Release Correlate with Disease Progression. We have analyzed the release of sIL-6Rα by ELISA in the supernatants of native myeloma cells and HMCLs (Fig. 1). All purified myeloma cell samples (n = 28) produced sIL-6Rα, but the production was heterogeneous, ranging from 28 pg/ml to 1607 pg/ml Of note, MM patients in

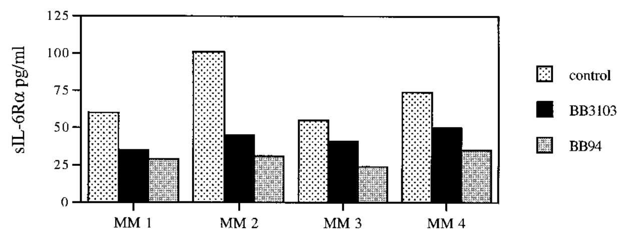

Fig. 3 Comparison of the inhibition of sIL-6Rα release induced by two different hydroxamate inhibitors. Myeloma cells were plated at the density of 10^6/ml in the presence of 25 μM BB-3103 or 25 μM BB-94, as indicated. After a 24-h incubation period, supernatants were collected and used for sIL-6Rα determination.

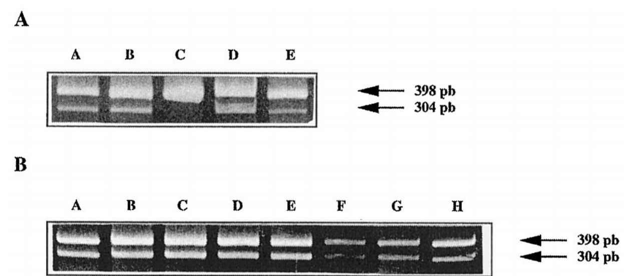

Fig. 4 Detection of the alternatively spliced mRNA for IL-6Rα in myeloma cells. The position of the 398-bp and 304-bp fragments corresponding to the mRNA of membrane IL-6Rα and to the alternatively spliced mRNA for IL-6Rα, respectively, are shown. *A*, native myeloma cells. *B*, HMCLs. *Lane A*, XG-1; *Lane B*, XG-2; *Lane C*, XG-6; *Lane D*, OPM-2; *Lane E*, LP1; *Lane F*, U266; *Lane G*, RPMI-8226; *Lane H*, NCI-H929.

relapse ($n = 11$) release more sIL-6Rα than those at diagnosis ($n = 17$; median value 310pg/ml *versus* 124pg/ml; $P < .05$). Moreover, the release of sIL-6Rα by HMCLs ($n = 13$) was found to be strongly up-regulated compared with that of native myeloma cells, even at relapse (1520 pg/ml *versus* 310 pg/ml, $P < .05$; Fig. 1). These results demonstrate an up-regulation of the release of sIL-6Rα during tumoral expansion *in vivo* and then immortalization *in vitro*.

Shedding of the Membrane IL-6Rα Is in Part Responsible for the Release of sIL-6Rα from Myeloma Cells. To determine whether shedding of IL-6Rα occurred on freshly isolated myeloma cells, we have analyzed the effects of BB-3103, a hydroxamate-based metalloproteinase inhibitor (15), both on the constitutive and PMA-induced production of sIL-6Rα (Fig. 2; Ref. 18). We have analyzed the effects of PMA because the IL-6Rα shedding has been shown to be regulated by the protein kinase C activity (19). The addition of 25 μM BB-3103 induced a strong inhibition of sIL-6Rα production (mean, 50% ± 9%) in all of the cases evaluated ($n = 10$), indicating that shedding of the IL-6Rα represents an important mechanism *in vivo.* Fig. 2 shows the data obtained for four patients. Moreover, after stimulation of myeloma cells by PMA, we observed an increase of sIL-6Rα concentration in cell supernatants (mean, 77% ± 20; $n = 10$). The inhibition of PMA-induced sIL-6Rα release by BB-3103 was similar to that of the constitutive sIL-6Rα released with a mean of 51% ± 16%. Finally, in three experiments, we show that the inhibition induced by BB-3103 is closed to the one observed with another metalloproteinase inhibitor, the batimastat BB-94 (Fig. 3).

Detection of the sIL-6Rα mRNA, Which Lacks the Transmembrane Domain in Myeloma Cells. To detect RNA transcripts encoding both membrane IL-6Rα or sIL-6Rα resulting of alternative splicing, oligonucleotides that flank the transmembrane domain were used. These oligonucleotides generate two fragments: (*a*) a 398-bp fragment corresponding to the membrane IL-6Rα; and (*b*) a 304-bp fragment resulting of alternative splicing. The RNA transcripts obtained from purified myeloma cells ($n = 15$) were analyzed by PCR, and for all samples except one, the two types of transcripts were detected (Fig. 4). Indeed, in one case (Fig. 4*A*, *Lane* C), only a 398-bp fragment corresponding to the membrane IL-6Rα was observed. The same results were obtained on HMCLs ($n = 13$), where the two transcripts were always detected (Fig. 4*B*). These data indicated that in most of the cases, myeloma cells are able to produce sIL-6Rα generated by alternative splicing.

Discussion

IL-6 plays a central role in the survival and proliferation of myeloma cells and exerts its activity through either the membrane-bound IL-6Rα (CD126) or its interaction with the sIL-6Rα, which has the particularity to exhibit an agonistic activity. We and others have shown that a large proportion of myeloma cells express the IL-6Rα at the cell membrane (20, 21). Here, we demonstrate that all native myeloma cells and HMCLs release sIL-6Rα. Moreover, we show that the levels of sIL-6Rα release correlate with disease progression, the sIL-6Rα release being significantly up-regulated during tumoral expansion *in vivo* and immortalization *in vitro*. Our study of the mechanisms of sIL-6Rα release by purified malignant plasma cells demonstrate a proteolytic cleavage of cell surface IL-6Rα, as well as the presence of IL-6Rα mRNA transcripts lacking the transmembrane domain. These data confirm that generation of the sIL-6Rα is controlled by both shedding and alternative splicing on myeloma cells, as described for other cell types (22). The correlation of sIL-6Rα release with disease activity can be, in part, explained by the strong IL-6Rα expression on cell surface at the late stages of the disease (21). The shedding of the IL-6Rα is inhibited by a hydoxamate-based metalloproteinase inhibitor, suggesting that an endogeneous membrane metalloproteinase is responsible for this shedding. A same process has been involved in the release of active tumor necrosis factor from its membrane-bound form, and two metalloproteinases (*i.e.,* TACE and ADAM 10) responsible for this shedding have been identified (23–25). These two metalloproteinases are members of the ADAM family. Thus, it is reasonable to hypothesize that the metalloproteinase responsible for IL-6Rα shedding could be also a member of the ADAM protease family and that myeloma cells express it constitutively. It is noteworthy that different ADAM proteins of unknown function have been identified in myeloma cells (26).

The generation of sIL-6Rα seems to be regulated and more particularly in MM, where elevated serum sIL-6Rα levels have been detected in correlation with disease progres-

sion (7). It is conceivable that both mechanisms of generation of sIL-6Rα are regulated. Indeed, it has been shown that in human hepatoma cells, OSM regulates the release of sIL-6Rα through an alternative splicing (27). In the other way, the shedding must be regulated because it is limited by the activity of protein kinase C (18). Moreover, we can not exclude that the sheddase activity could be up-regulated during disease progression. Finally, a physiological inducer of the IL-6Rα shedding (*i.e.,* the C-reactive protein) has been described recently (28). C-reactive protein, which is overproduced in MM in response to IL-6, is an important prognostic factor. Also, this physiological inducer of the shedding of IL-6Rα could be of interest in MM and could explain the increase of sIL-6Rα release at relapse. The regulation of the different mechanisms of generation of the sIL-6Rα are currently under investigation in myeloma cells. The fact that sIL-6Rα potentiates the effect of IL-6 and our findings showing that sIL-6Rα is enhanced in MM patients at relapse suggest that sIL-6Rα could be an interesting therapeutical target in this disease. Because we show that inhibitors of metalloproteinases could interfere in the process of sIL-6Rα generation, the use of metalloproteinase inhibitors could be a new therapeutical approach in MM. We had previously underlined the interest of using such synthetic metalloproteinase inhibitors in MM because they could counteract the increase of matrix metalloproteinase activity observed in MM (29). Moreover, some synthetic inhibitors, and in particular the BB-94 (Batimastat), are now available for clinical trials (30–32). In conclusion, we have shown that myeloma cells themselves could release sIL-6Rα, an agonist of IL-6, in correlation with tumor progression. This tumoral production and clinical correlation could explain the increase of serum sIL-6Rα levels and its prognostic value in MM. By inducing (2, 33), rather than secreting, IL-6 and releasing sIL-6Rα, myeloma cells can generate IL-6/sIL-6Rα complex (*i.e.,* super IL-6), able to promote myeloma cell growth and bone destruction. By inhibiting sIL-6Rα shedding as shown here, metalloproteinase inhibitors could represent a new therapeutical alternative in MM.

Acknowledgments

We thank Drs. P. Brown and H. Mills (British Biotech, Ltd.) for supplying BB-3103 and BB-94 and M. Etrillard for technical assistance.

References

1. Kawano, M., Hirano, T., Matsuda, T., Taga, T., Horii, Y., and Iwato, K. Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. Nature (Lond.), *332:* 83–85, 1988.

2. Klein, B., Zhang, X., Jourdan, M., Content, J., Houssiau, F., Aarden, L., Piechaczyk, M., and Bataille, R. Paracrine rather than autocrine regulation of myeloma cell growth and differenciation by interleukin-6. Blood, *73:* 517, 1989.

3. Kishimoto, T., Akira, S., Narazaki, M., and Taga, T. Interleukin-6 family of cytokines and gp130. Blood, *86:* 1243–1254, 1995.

4. Peters, M., Jacobs, S., Ehlers, M., Vollmer, P., Müllberg, J., Wolf, E., Brem, G., Meyer, K., and Rose-John, S. The function of the soluble interleukin 6 (IL-6) receptor *in vivo*: sensitization of human soluble IL-6 receptor transgenic mice toward IL-6 and prolongation of the plasma half-life of IL-6. J. Exp. Med., *183:* 1399–1406, 1996.

5. Schirmacher, P., Peters, M., Ciliberto, G., Blessing, M., Lotz, J., Büschenfelde, M. Z., and Rose-John, S. Hepatocellular hyperplasia, plasmacytoma formation, and extramedullary hematopoiesis in interleukin (IL)-6/soluble IL-6 receptor double-transgenic mice. Am. J. Pathol., *153:* 639–648, 1998.

6. Tamura, T., Udagawa, N., Takahashi, N., Miyaura, C., Tanaka, S., Yamada, Y., Koishihara, Y., Ohsugi, Y., Kumaki, K., Taga, T., Kishimoto, T., and Suda, T. Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6. Proc. Natl. Acad. Sci. USA, *90:* 11924–11928, 1993.

7. Gaillard, J., Bataille, R., Brailly, H., Zuber, C., Yasukawa, K., Attal, M., Maruo, N., Taga, T., Kishimoto, T., and Klein, B. Increased and highly stable levels of functional soluble interleukin-6 receptor in sera of patients with monoclonal gammopathy. Eur. J. Immunol., *23:* 820–824, 1993.

8. Stasi, R., Brunetti, M., Parma, A., Giulio, C., Terzoli, E., and Pagano, A. The prognostic value of soluble interleukin-6 receptor in patients with multiple myeloma. Cancer (Phila.), *82:* 1860–1866, 1998.

9. Gaillard, J., Liautard, J., Klein, B., and Brochier, J. Major role of the soluble interleukin-6/interleukin-6 receptor complex for the proliferation of interleukin-6-dependent human myeloma cell lines. Eur. J. Immunol., *27:* 3332–3340, 1997.

10. Diamant, M., Hansen, M., Rieneck, K., Svenson, M., Yasukawa, K., and Bendtzen, K. Differential interleukin-6 (IL-6) responses of three established myeloma cell lines in the presence of soluble human IL-6 receptors. Leukemia Res., *20:* 291–301, 1996.

11. Müllberg, J., Durie, F., Otten-Evans, C., Alderson, M., Rose-John, S., Cosman, D., Black, R., and Mohler, K. A metalloprotease inhibitor blocks shedding of the IL-6 receptor and the p60 TNF receptor. J. Immunol., *155:* 5198–5205, 1995.

12. Hargreaves, P., Wang, F., Antcliff, J., Murphy, G., Lawry, J., Graham, R., Russell, G., and Croucher, P. Human myeloma cells shed the interleukin-6 receptor: inhibition by tissue inhibitor of metalloproteinase-3 and a hydroxamate-based metalloproteinase inhibitor. Br. J. Haematol., *101:* 694–702, 1998.

13. Horiuchi, S., Koyanagi, Y., Zhou, Y., Miyamoto, H., Tanaka, Y., Waki, M., Matsumoto, A., Yamamoto, M., and Yamamoto, N. Soluble interleukin-6 receptors released from T cell or granulocyte/macrophage cell lines and human peripheral blood mononuclear cells are generated through an alternative splicing mechanism. Eur. J. Immunol., *24:* 1945–1948, 1994.

14. Lust, J., Donovan, K., Kline, M., Greipp, P., Kyle, R., and Maihle, N. Isolation of an mRNA encoding a soluble form of the human interleukin-6 receptor. Cytokine, *4:* 96–100, 1992.

15. Ancuta, P., Fahmi, H., Pons, J., Blay, K. L., and Chaby, R. Involvement of the membrane form of tumor necrosis factor-α in lipopolysaccharide-induced priming of mouse peritoneal macrophages for enhanced nitric oxide response to lipopolysaccharide. Immunology., *92:* 259–266, 1997.

16. Pellat-Deceunynck, C., Bataille, R., Robillard, N., Harousseau, J., Rapp, M., Juge-Morineau, N., Wijdenes, J., and Amiot, M. Expression of CD28 and CD40 in human myeloma cells: a comparative study with normal plasma cells, Blood, *84:* 2597–2603, 1994.

17. Durie, B., and Salmon, S. Multiple myeloma, macroglobulinaemia and monoclonal gammopathies. *In:* A. V. Hoffbrand, M. C. Brain, and J. Hish (eds.), Recent Advances in Haematology, pp. 243–261. Edinburgh Churchill Livingstone, 1977.

18. Müllberg, J., Schooltink, H., Stoyan, T., Heinrich, P., and Rose-John, S. Protein kinase C activity is rate limiting for shedding of the interleukin-6 receptor. Biochem. Biophys. Res. Commun., *189:* 794–800, 1992.

19. Müllberg, J., Schooltink, H., Stoyan, T., Günther, M., and Graeve, L. The soluble interleukin-6 receptor is generated by shedding. Eur. J. Immunol., *23:* 473–480, 1993.

20. Hata, H., Xiao, H., Petrucci, M., Woodliff, J., Chang, R., and Epstein, J. Interleukin-6 gene expression in multiple myeloma: a characteristic of immature tumor cells. Blood, *81:* 3357–3364, 1993.

21. Barillé, S., Thabard, W., Robillard, N., Pineau, D., Harousseau, J-L., Bataille, R., and Amiot, M. CD130 rather than CD126 expression is associated with disease activity in multiple myeloma. Br. J. Haematol., in press, 1999.

22. Gallea-Robache, S., Morand, V., Millet, S., Bruneau, J., Bhatnagar, N., Chouaib, S., and Roman-Roman, S. A metalloproteinase inhibitor blocks the shedding of soluble cytokine receptors and processing of transmembrane cytokine precursors in human monocytic cells. Cytokine, *9:* 340–346, 1997.

23. Black, R., Rauch, C., Kozlosky, C., Peschon, J., Slack, J., Wolfson, M., Castner, B., Stocking, K., Reddy, P., Srinivasan, S., Nelson, N., Boiani, N., Schooley, K., Gerhart, M., Davis, R., Fitzner, J., Johnson, R., Paxton, R., March, C., and Cerretti, D. A metalloproteinase disintegrin that releases tumor-necrosis factor-α from cells. Nature (Lond.), *385:* 729–733, 1997.

24. Moss, M., Jin, S., Milla, M., Burkhart, W., Carter, H., Chen, W., Clay, W., Didsbury, J., Hassler, D., Hoffman, C., Kost, T., Lambert, M., Leesnitzer, M., Cauley, P., Geehan, G., Mitchell, J., Moyers, M., Pahel, G., Rocque, W., Overton, L., Schoenen, F., Seaton, T., Su, J., Warner, J., Willard, D., and Becherer, J. Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-α. Nature (Lond.), *385:* 733–736, 1997.

25. Rosendhal, M., Ko, S., Long, D., Brewer, M., Rosenzweig, B., Hedl, E., Anderson, L., Pyle, S., Moreland, J., Meyers, M., Kohno, T., Lyons, D., and Lichenstein, H. Identification and characterization of a pro-tumor necrosis factor-α-processing enzyme from the ADAM family of zinc metalloprotease. J. Biol. Chem., *272:* 24588–24593, 1997.

26. McKie, N., Dallas, D., Edwards, T., Apperley, J., and Graham, R. Cloning of a novel membrane-linked metalloproteinase from human myeloma cells. Biochem. J., *318:* 459–462, 1996.

27. Cichy, J., Rose-John, S., Potempa, J., Pryjma, J., and Travis, J. Oncostatin M stimulates the expression and release of the IL-6 receptor in human hepatoma HepG2 cells. J. Immunol., *159:* 5648–5653, 1997.

28. Jones, S., Novick, D., Horiuchi, S., Yamamoto, N., Szalai, A., and Fuller, G. C-reactive proteins: a physiological activator of interleukin-6 receptor shedding. J. Exp. Med., *189:* 599–604, 1999.

29. Barillé, S., Akhoundi, C., Collette, M., Mellerin, M.-P., Rapp, M.-J., Harousseau, J.-L., Bataille, R., and Amiot, M. Metalloproteinases in multiple myeloma: production of matrix metalloproteinase-9 (MMP-9), activation of proMMP-2, and induction of MMP-1 by myeloma cells. Blood, *90:* 1649–1655, 1997.

30. Brown, P., and Giavazzi, R. Matrix metalloproteinase inhibition: a review of anti-tumor activity. Ann. Oncol., *6:* 967, 1995.

31. Beattie, G., and Smyth, J. Phase I study of intraperitoneal metalloproteinase inhibitor BB94 in patients with malignant ascites. Clin. Cancer Res., *8:* 1899–1902, 1998.

32. Parsons, S., Watson, S., and Steele, R. Phase I/II trial of batimastat, a matrix metalloproteinase inhibitor, in patients with malignant ascites. Eur. J. Surg. Oncol., 526–531, 1997.

33. Barillé, S., Collette, M., Bataille, R., and Amiot, M. Myeloma cells up-regulate IL-6 secretion in osteoblatic cells through cell-to-cell contact but down-regulate osteocalcin. Blood, *90:* 1649–1655, 1997.