Haematologica 1998; 83:298-304

original paper

bcl-2 expression in plasma cells from neoplastic gammopathies and reactive plasmacytosis: a comparative study

Alicia Miguel-García, Teresa Orero, Estela Matutes,* Francisco Carbonell,° Amparo Miguel-Sosa, Mariano Linares, Fabian Tarín, Magdalena Herrera,# Juan García-Talavera,# Felix Carbonell-Ramón

*Service of Hematology, Valencia University General Hospital, Valencia, Spain. *Academic Hematology, Royal Marsden Hospital, London, UK; °Valencia Transfusion Center, Valencia, Spain; #Service of Hematology, Hospital de la Candelaria, Santa Cruz de Tenerife, Spain*

Abstract

Background and Objective. **bcl-2 oncoprotein plays a major physiological role in hemopoietic and non-hemopoietic cells by preventing apoptosis (programmed cell death). Disregulation of this process may be important in oncogenesis and the response to treatment of patients with different hematological malignancies. We have investigated the levels of bcl-2 expression in plasma cells from patients with reactive plasmacytosis (RP), monoclonal gammopathy of unknown significance (MGUS) and multiple myeloma (MM), correlating the bcl-2 expression and clinico-biological features in MM patients.**

Design and Methods. **The percentage of bcl-2 (+) plasma cells and levels of bcl-2 protein expression were investigated in 73 patients at diagnosis. Immunofluorescence and immunoenzymatic methods were applied using McAb against bcl-2 protein, and the intensity of protein expression was assessed by both the mean channel fluorescence intensity (MFI) and semiquantitative methods. To evaluate the intensity of bcl-2 expression in proliferating plasma cells, sequential double immunoenzymatic staining with McAb Ki-67 and bcl-2 was applied in 10 patients with MM. Correlations between bcl-2 expression and the clinico-biological features in MM patients were also studied.**

Results. **The proportion of bcl-2 (+) plasma cells was significantly higher in MGUS and MM than in RP ($p<0.001$). The intensity of bcl-2 expression in plasma cells (assessed by MFI) was significantly different between all groups studied ($p<0.0001$). RP showed lower expression than MGUS and MM patients. MM stage III patients demonstrated higher bcl-2 expression values than MGUS ($p<0.01$). According to the proportion of plasma cells expressing Ki-67, patients with a proliferative index (Ki-67$^+$) > 4% showed lower bcl-2 expression than patients with proliferative index < 4% ($p<0.05$). Immunocytochemistry showed that plasma cells from RP had a lower intensity of bcl-2 expression than MM ($p<0.001$), and double immunostaining Ki-67/bcl-2 demonstrated that the majority of proliferating plasma cells had weak bcl-2 expression. There was no correlation between bcl-2 expression and clinico-biological parameters, response to therapy or overall survival in MM patients.**

Interpretation and Conclusions. **Globally, the number of bcl-2 (+) plasma cells and the intensity of protein expression in neoplastic gammopathies are significantly higher than in reactive plasmacytosis and bcl-2 levels tend to increase with disease stage. bcl-2 may be relevant to the pathogenesis of malignant gammopathies, prolonging the survival of plasma cells by preventing apoptosis and increasing the chance of acquiring additional gene defects. bcl-2 expression could also contribute to the resistance to chemotherapy observed in MM disease.**

©1998, Ferrata Storti Foundation

Key words: bcl-2, plasma cells, monoclonal gammopathy of unknown significance, multiple myeloma, apoptosis

Correspondence: Alicia Miguel-García, MD, Centro de Transfusión de Valencia, Avda. del Cid 65, Valencia, Spain. Phone: international + 34-6- 3868112 • Fax: international + 34-6-3868109.

The bcl-2 proto-oncogene was originally discovered and mapped to chromosome 18q21 in a follicular lymphoma with t(14;18)(q32;q21).[1,2] In this lymphoma, the bcl-2 gene rearranges with 14q32 where the Ig heavy chain gene is located, resulting in the t(14;18)(q32q21) – a hallmark of this lymphoma. This rearrangement leads to the activation and over production of otherwise normal bcl-2 protein, which, though characteristic, is not specific of lymphomas bearing t(14;18).[3]

Posteriorly, it was shown that the bcl-2 protein plays a major physiological role in haemopoietic and non hemopoietic cells[4-6] by allowing cells to survive by preventing the triggering of apoptosis (programmed cell death)[7] a normal process by which cells are eliminated during embryogenic development and in adult life. Disruption of this normal physiological pathway results in illegitimate cell survival and may increase the chances for cells to acquire additional gene defects that promote aberrant growth and proliferation, thus facilitating cancer development.[8-10] For instance, deregulated bcl-2 expression prolongs the survival of some interleukin-dependent hematopoietic cell lines when deprived of growth factors.[11]

© Ferrata Storti Foundation

Recent *in vitro* studies of murine and human leukemia cell lines have demonstrated that, although the suppression of cell proliferation is not prevented, high levels of bcl-2 protein may protect cells from undergoing apoptosis in the presence of glucocorticoids and a variety of chemotherapeutic drugs.[12] These findings suggest that disregulation of bcl-2 protein expression may play an important role in the oncogenesis and response to treatment of patients with different hematological malignancies other than follicular lymphoma. For instance, the bcl-2 protein has been shown to be expressed in blast cells from acute leukemia patients and the levels of expression seem to be related to a poor outcome.[13-16] Similarly, cells from most mature B-cell disorders, including follicular lymphoma,[17,18] diffuse large cell lymphoma[19-21] and chronic lymphocytic leukemia[22,23] express bcl-2 at high levels though the relationship between bcl-2 expression and prognosis has not been established.

bcl-2 is also expressed in neoplastic cells from malignancies of B cells in the late stages of differentiation, such multiple myeloma (MM) and myeloma cell lines,[24-26] and recent studies have suggested that chemotherapeutic agents may enhance bcl-2 expression in such neoplastic plasma cells and contribute to chemo-resistance.[27] However, a correlation between the levels of bcl-2 expression, clinical features at presentation in MM and outcome of the disease has not been previously investigated.

We have studied the clinical significance, if any, of the levels of bcl-2 protein expression in plasma cells from multiple myeloma (MM) by: a) comparing results between plasma cells from patients with reactive plasmacytosis (RP) and monoclonal gammopathy of unknown significance (MGUS) and MM, and b) correlating within MM the intensity of bcl-2 protein expression to the clinical-biological features, response to treatment and survival.

Materials and Methods

Patients

Seventy-three patients were studied at diagnosis. This series included: 12 cases of reactive plasmacytosis (RP), 12 monoclonal gammopathies of unknown significance (MGUS), and 49 multiple myeloma (MM). The main clinical features were:

- *for reactive plasmacytosis* (RP): 6 males and 6 females (M/F:1), mean age 61 years (range 30-88). The diagnosis was based on bone marrow infiltration by polyclonal plasma cells as assessed by Ig light chain restriction (>5%). The underlying pathologies in these patients were connective tissue disease (n=5), cancer (n=5) and AIDS(n=2);
- *monoclonal gammopathy of unknown significance* (MGUS): the M/F ratio was 1, with a mean age of 72 years (range 65-86). The diagnosis was made according to Kyle's criteria:[28] all patients had a serum monoclonal M band < 3 g/dL and bone marrow infiltration by plasma cells was less than 10%;
- *multiple myeloma* (MM): the clinico-biological characteristics are shown in Table 1. There were 25 males and 24 females (M/F:1) with mean age 69 years (range 39-87). The diagnosis was based on the criteria of the *Chronic Leukemia-Myeloma Task Force.*[29] Stages according to Durie-Salmon's criteria[30] were: stage I (n=2), stage II (n=10), and stage III (n=37); this latter group included two patients with plasma cell leukemia. The serum M band was IgG in 27 patients, and IgA in 15; 7 patients had only light chains in urine (Bence-Jones$^+$) (κ^+ 4, λ^+ 3). The evaluated prognostic factors associated to outcome were: age, tumor stage (according to Durie and Salmon),[30] performance status (according to the *Eastern Cooperative Oncology Group* scale, ECOG), bone lytic lesions, percentage of bone marrow plasma cells, Ki-67 expression in bone marrow plasma cells, M-band Ig class , haemoglobin, calcium and creatinine serum levels, LDH, C reactive protein (CRP), M-protein levels and β_2 microglobulin. Thirty patients with advanced (II-IIII) stages and over 65 years of age were treated with melphalan-prednisolone (73%), response being evaluated after 6 cycles. Eleven patients (27%) aged < 65 years were treated with combination chemotherapy VCMP/ VBAP (vin-

©Ferrata Storti Foundation

Table 1. Clinico-biological characteristics of multiple myeloma patients (n=49).

	Mean±SD	*Range*
Age (years)	69	39-87
Hemoglobin (g/dL)	9.62±0.34	4-13.5
Bone marrow infiltration (%)	46.96±4.13	15-100
Creatinine (mg/dL)	1.6±0.21	0.5-7.1
Calcium (mg/dL)	10.04±0.2	8.08-14.4
LDH (U/L)	385.07±62.11	77-2910
β_2 microglobulin (ng/L)	6,181.05±899	1,462-33,000
CRP (mg/L)	3.75±1.45	0.09-7
Monoclonal component (mg/dL)		
IgG (n=27)	527.7±478.2	1,900-10,700
IgA (n=15)	4,958.3±597.6	1,600-9,320
Bence-Jones (n=7) (g/24 h)	9.37±3.8	0.7-45
Ki-67 (%) (plasma cells)	3.99±1.08	0-40
	Nº cases	*%*
Performance status (ECOG)		
0-1	28	58
2-4	21	42
Bone lytic lesions		
0	8	17
1	13	27
2	13	27
3	15	29

cristine, cyclophosphamide, melphalan, prednisone/vincristine, BCNU, adriamycin and prednisone) and response was assessed after four cycles. Clinical response was assessed according to the *Leukemia-Myeloma Task Force* criteria,[29] as follows: M band reduction of at least 50% and/or a 75% decrease in Bence-Jones protein without any increase in the size or number of bone lytic lesions. Data concerning response to treatment and follow-up were available and analyzed in 30 patients.

Methods

Mononuclear cells were isolated from heparinized bone marrow specimens by Ficoll-Hypaque density gradient centrifugation. Cell reactivity with the various monoclonal antibodies (MoAb) was analyzed by flow cytometry and by immunocytochemistry on cytospin made slides. The McAb used were fluorescein-conjugated bcl-2 (bcl-2 FITC) (Dakopatts), unconjugated bcl-2 (Dakopatts), CD38 phycoerythrin conjugate (CD38PE), CD45 PerCP (Becton-Dickinson) and unlabeled Ki-67 (Dakopatts).

Immunofluorescence study

Samples (5×10^6 cells/mL) were subjected to a simultaneous triple labeling as described below. Cells were simultaneously incubated for 10 min in the dark with the CD38 PE and CD45 PerCP MoAb, in order to identify plasma cells. After two washes with phosphate buffer saline (PBS), cells were further incubated for 10 min with FACS lysis solution (1:10 distilled water), to permeabilize the cell membrane for the detection of cytoplasmic antigens. After two washes with PBS, the cells were again incubated for 10 min with FITC bcl-2 and washed two more times with PBS. All samples were acquired on the FACscan flow cytometer and analyzed using "*Paint-a-gate*" software, with at least 5000 events being evaluated for each sample. Plasma cells were identified by their forward-side scatter properties and strong reactivity with CD38. The intensity of bcl-2 protein expression was assessed by the mean channel fluorescence intensity (MFI) value of plasma cells positive with bcl-2. In order to standardize the method, a ratio between the MFI value of the positive plasma and non-plasma cells was established in each sample.

Immunoenzymatic study

Immunocytochemistry was performed on bone marrow mononuclear cells by the alkaline phosphatase-antialkaline phosphatase method (APAAP)[31] to assess the cytoplasmic expression of bcl-2 in cells displaying the morphology of plasma cells. Cytospin made slides were examined under ×100 optic microscope magnification, and at least 100 plasma cells were evaluated in each sample. The intensity of protein expression was assessed semiquantitatively as follows: 0 (negative), + (1, weak), ++ (2, moderate) and +++(3, strong) staining. No cut-off point was used for either the percentage of bcl-2 positive cells or for the degree of intensity of bcl-2 expression. Normal peripheral blood lymphoid cells were used as bcl-2 (+) controls. In patients with MM, the degree of proliferation in plasma cells (proliferative index) was investigated by nuclear staining with the MoAb Ki-67, using an immunoperoxidase (IP) sandwich technique.[32] Sequential double immunoenzymatic staining IP/APAAP [33] with Ki-67 and bcl-2 was applied in 10 patients with MM stage III to evaluate the intensity of bcl-2 expression in the proliferating cells (Ki-67 positive).

Statistical analysis

Comparisons of bcl-2 expression between the diagnostic groups were made with the Kruskall-Wallis test. Multiple comparisons between each pair of groups were performed by the Bonferroni method.

The relation between clinical and biological features in MM and bcl-2 expression was investigated by univariate analysis: a) comparisons of bcl-2 expression between strata for qualitative variables were made using the Mann-Whitney U-test or the Kruskall-Wallis test; b) relations between bcl-2 expression and quantitative variables were analyzed by simple linear correlation.

Survival curves were estimated by the Kaplan and Meier method[34] and were compared by the log-rank test.[35] All analyses were performed with the Statistical Package for the Social Sciences (SPSS) software (SPSS Inc., Chicago).

Results

bcl-2 expression in normal and neoplastic plasma cells

Flow cytometry. The proportion of bcl-2^+ plasma cells in the various groups ranged from 48 to 98%, and was significantly lower ($p<0.001$) in PR than in MGUS and MM at all stages: stage I+II (94%,) and stage III (93%) (Table 2). There were no significant differences between patients with MGUS and MM.

The intensity of bcl-2 expression in plasma cells is represented in Figure 1A. The intensity of bcl-2 expression differed significantly among all groups studied ($p<0.00001$), as follows: RP had significantly ($p<0.0001$) lower ratios (0.88±0.17, range 0.6-1.2) than MGUS (1.36±0.27, range 1-1.9), MM stages I+II (1.51±0.4, range 1-2.04; $p<0.0001$) and MM stage III (2.06±0.78, range 1-4; $p<0.0001$). Differences were not significant when comparing MGUS and MM stages I+II, or early MM stages (I+II) and advanced MM stage (III). bcl-2 expression was significantly lower ($p<0.01$) in MGUS than in MM stage III patients.

Immunocytochemistry. The proportion of bcl-2^+ plasma cells was significantly higher ($p<0.001$) in MGUS (96%, range 90-100), MM I+II (98%, range 90-100) and MM III (96%, range 80-100) than in RP (48%, range 24-90) (Table 2). There were also significant

© Ferrata Storti Foundation

Table 2. Percentage of bcl-2 positive plasma cells.

	RP	*MGUS*	*MM (I+II)*	*MM(III)*
% bcl-2⁺	48±23*	97±4	98±3	96±6
IC	(24-90)	(90-100)	(90-100)	(80-100)
	n=12	n=12	n=12	n=27
% bcl-2⁺	57±20*	94±7	94±9	93±14
IF	(25-81)	(80-100)	(76-100)	(47-100)
	n=9	n=11	n=12	n=37

*Values are given as mean±SE. Abbreviations: IC, immunocytochemistry. IF, immunofluorescence. RP, reactive plasmocytosis. MGUS: monoclonal gammopathy of unknown significance. MM (I+II), multiple myeloma stages I and II. MM (III), multiple myeloma stage III. n= number of patients. *significant differences between RP and the rest of groups (p<0.001).*

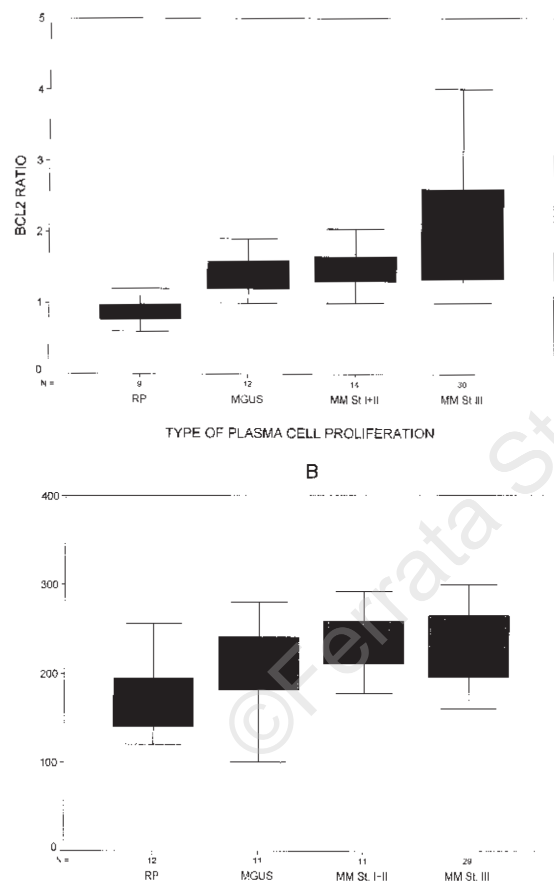

Figure 1. Comparison of the intensity of bcl-2 expression between reactive plasmocytosis (RP), monoclonal gammopathy of unknown significance (MGUS), multiple myeloma stages I and II (MM st. I+II) and MM stage III. Each group of patients is displayed as a box plot: the box covers the range between the first and the third quartile and the central is at the median. The whiskers (???) extend to the more extreme.
A: Flow cytometric results are expressed as the ratio between the MFI (mean fluorescence intensity channel) of bcl-2 in plasma and non plasma cells.
B: Immunocytochemistry results are expressed as a scoring system based on the staining intensity.

Table 3. Intensity of bcl-2 expression in Ki-67 positive plasma cells.

	% bcl-2⁺/Ki-67⁺	% bcl-2⁺⁺/⁺⁺⁺/Ki-67⁺
1.	62	38
2.	83	17
3.	70	30
4.	86	14
5.	85	15
6.	98	2
7.	83	17
8.	100	0
9.	64	36
10.	93	7
mean	8.4*	17.6*
	(62-100)	(0-38)

Double immunostaining: Ki-67 (IP)/bcl-2 (APAAP).
(+)= weak staining. (++/+++)= moderate / strong staining.
(*) significant differences between both groups (p<0.0001)

©Ferrata Storti Foundation

differences in the intensity of bcl-2 expression between RP (174(43, range 120-256) and MM (stage I+II: 237(36, range 178-292; stage III: 232(41, range 160-300) (Figure 1B) (p<0.001).

Double immunostaining with Ki-67 and bcl-2 showed that the majority of proliferating MM plasma cells (82±4%) had weak (+) bcl-2 expression while the remaining proliferating cells (18±4%) exhibited moderate (++) or, rarely, strong (+++) expression (p<0.0001) (Table 3).

Relation of bcl-2 expression to prognostic factors, treatment outcome and survival in MM

Correlation between bcl-2 expression and prognostic factors in MM. There were no significant differences in mean values of bcl-2 expression intensity between groups of qualitative variables (performance status, osteolysis and M band Ig class). No correlation was observed on applying a univariate analysis between the intensity of bcl-2 expression (MFI ratio) and the various quantitative variables: age (r=0.15, p=0.39), hemoglobin level (r=0.12, p=0.19), creatinine (r=0.12, p=0.49), LDH (r=0.01, p=0.49), CRP (r=0.15, p=0.40), M-protein levels (r=0.27, p=0.48), β_2 microglobulin (r=0.00, p=0.98) and Ki-67 expression in bone marrow plasma cells (r=0.004, p=0.82). Only the percentage of bone marrow plasma cells (r=0.59, p<0.05) and serum calcium levels (r=45, p<0.05) showed a minimal correlation.

Relationship between proliferative index and bcl-2 expression in MM plasma cells. According to the proportion of plasma cells expressing Ki-67, cases were divided into two groups with more or less than 4% of Ki-67⁺ cells. Patients with proliferative index < 4% exhibited greater bcl-2 expression (bcl-2 ratio=1.96±0.19) than patients with proliferative index (4% (bcl-2 ratio=1.53±0.12)

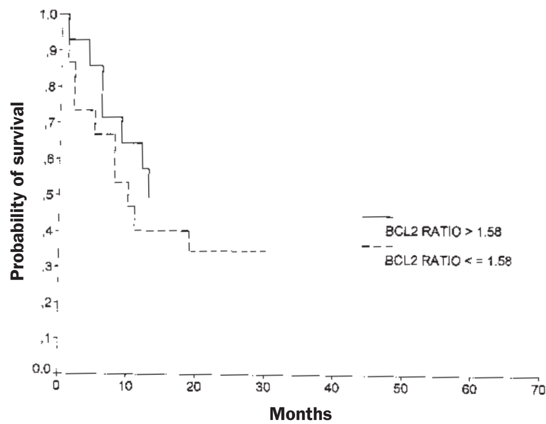

Figure 2. Survival of patients with multiple myeloma with high bcl-2 ratio (>1.58, patients 15, median 10 months) and low bcl-2 ratio (≤ 1.58, patients 15, median 10 months). The difference was not significant (log rank test: p=0.39).

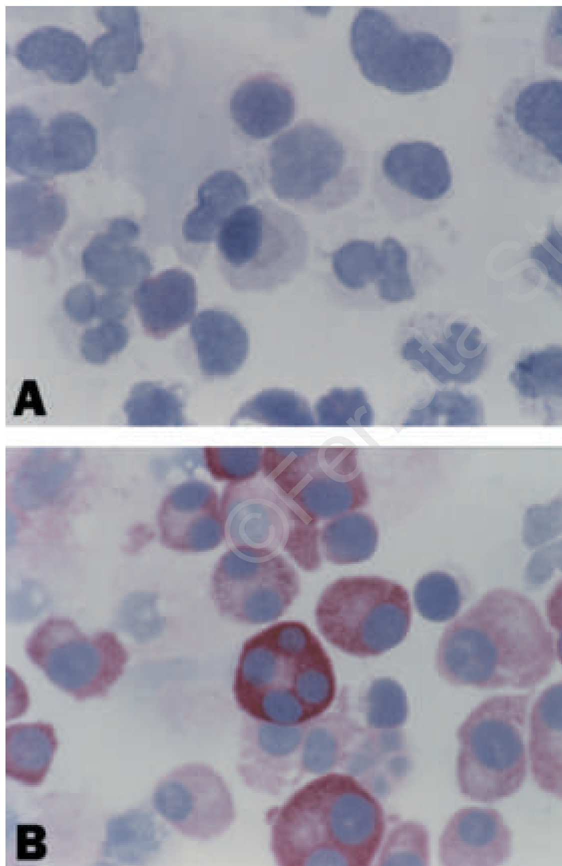

Figure 3. Bone marrow cytospin showing several bcl-2 positive plasma cells (red cytoplasmic stain, APAAP technique) with differences in the intensity of bcl-2 expression from a patient with multiple myeloma (3A); a bcl-2 negative plasma cell from a patient with reactive plasmocytosis, coexisting with bcl-2 positive lymphoid cells (3B).

($p<0.05$).

Correlation between bcl-2 expression and treatment response in MM. Response to treatment (MP or VCMP/VBAP) was evaluated in 30 patients. No significant differences were found in the intensity of bcl-2 expression (MFI ratio) between responders (16 patients; 1.98±0.97) and non responders (14 patients; 1.94±0.81)(data not shown).

Correlation between bcl-2 expression and survival in MM: The median follow-up in 30 MM patients was 12(3 months (range, 0.5-69 months). Seventeen patients died (two during the first month after the diagnosis) and the remainign 13 are still alive.

Two groups of patients according to median bcl-2 expression were analyzed. Patients with a bcl-2 ratio lower than the median (1.58) had a median survival of 10±3 months while patients with ratios higher than 1.58 had a median survival of 13 months. Although the follow-up was too short, a trend towards a more

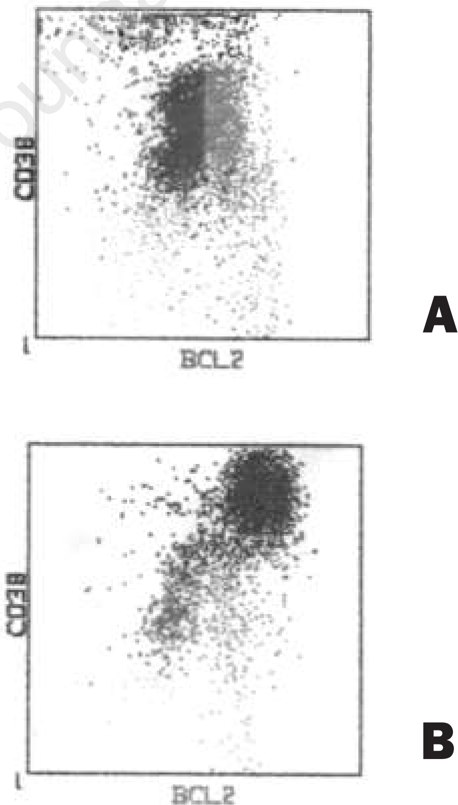

Figure 4. Bone marrow mononuclear cells stained with CD38-PE and bcl-2 FITC, acquired on the FACscan flow cytometer and analyzed using *Paint-a-gate* software. 4A: plasma cells ($CD38^{+++}$) from a patient with reactive plasmocytosis, 60% of them being bcl-2 negative. The intensity of bcl-2 expression in positive plasma cells is similar to other bcl-2 positive mononuclear cells in the sample. 4B: plasma cells ($CD38^{+++}$) from a patient with multiple myeloma, 95% of them being bcl-2 positive and exhibiting a greater intensity of expression than other bcl-2 positive cells in the same sample.

©Ferrata Storti Foundation

prolonged survival among patients with higher levels of bcl-2 was observed; however, no significant differences in survival probability were found between patients with lower or higher- than -median bcl-2 values (log-rank test p>0.05) (Figure 2).

Discussion

This study shows that patients with MGUS and MM have a significantly higher number of bcl-2+ plasma cells, and that they overexpress such protein compared to patients whose bone marrow presents an increase in non-clonal /reactive plasma cells (Figures 3 and 4). These findings are in contrast to other reports where no differences between reactive and clonal plasma cells were found in terms of bcl-2 expression,[24-26] though in these studies other methods were used and very few samples were analyzed. In addition, we observed that bcl-2 expression tends to increase parallel to disease stage: thus, while MGUS exhibited the lowest levels, MM in advanced stage (III) had the greatest expression (p<0.01), and early MM stages (I/II) presented intermediate levels; no statistical differences were found between MM stage I/II and MGUS or MM stage III. However, although a larger number of cases must be analyzed, bcl-2 overexpression in MM might contribute to disease progression by increasing the chances of acquiring secondary genetic changes in an already established neoplastic clone.

As in other hemopoietic malignancies, bcl-2 expression in MM seems to be inversely correlated to the proliferative rate of the tumor, as shown by staining with Ki-67.[36-38] Although the reason for this is not known, it could be related to the fact that bcl-2 delays cell enter to cycle.[39]

The clinical impact of bcl-2 expression in hemopoietic malignancies is variable and controversial. For instance, while in AML it seems to be correlated to drug resistance,[11,12] the significance of bcl-2 in ALL[13,14] is little or none. In mature lymphoproliferative disorders, a number of studies have shown that bcl-2 overexpression is related to resistance therapy and/or worst survival in B-NHL[18,19,40] and CLL;[41] few studies have been unable to confirm this correlation.[17,39]

The few studies available on MM have documented that bcl-2 does not play a major role as a factor influencing the response to treatment and/or survival.[42] The present study confirms such findings, as we could not correlate the level of bcl-2 expression to any other clinical parameter known to have prognostic significance in MM or to patient response to therapy or overall survival, though a trend towards longer survival was observed among patients expressing higher levels of bcl-2. This finding may be related to the lower bcl-2 expression observed in patients with a higher proliferative index, considered an adverse prognostic factor in MM patients. Our findings, and those reported previously may be due in part to the different treatment modalities used, including those for low or high-grade malignancies, and to the fact that the patients are old and may die from causes unrelated to the disease.

To summarize, we have demonstrated that the percentage and levels of bcl-2 in plasma cells from neoplastic gammopathies are significantly higher than in reactive plasmacytosis, and that the intensity of bcl-2 expression tends to increase with disease stage. bcl-2 may play a role in the pathogenesis of malignant gammopathies, extending the survival of plasma cells (by protecting them from apoptosis) and increasing the chance for cells to acquire additional gene defects. Although other studies are required, bcl-2 levels could also be related to the resistance to chemotherapy observed in MM disease.

Contributions and Acknowledgments

AM-G, TO, AM-S formulated the design of the study and took part, developed and carried out all the assays. FC contributed to the immunofluorescence assay. EM took part in the conception of the study , interpretation and writing of the paper, and gave final approval of the version to be published. ML, FCR, FT, JG-T and MH contributed to the design of the study and were involved in the clinical assessment of the patients. ML contributed with the statistical analysis. AM-G was the main investigator and wrote the paper, though all authors contributed to the analysis and manuscript preparation.

The criteria for the order in which the names of the authors appear are based on their contribution to the design, analysis, interpretation of data and execution of the study.

Disclosures

Conflict of interest: none

Redundant publication: no substantial overlapping with previous papers.

Manuscript processing

Manuscript received September 29, 1997; accepted January 8, 1998.

©Ferrata Storti Foundation

References

1. Fukura S, Rowley JD, Variokojis D, Golomb HM. Chromosome abnormalities in poorly differentiated lymphocytic lymphoma. Cancer Res 1979; 39:3119-28.
2. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. Science 1984; 226:1097-9.
3. Ngan BY, Chen-levy Z, Weiss LM, Warke RA, Cleary ML. Expression in non-Hodgkin's lymphoma of the bcl-2 protein associated with the t(14;18) translocation. N Engl J Med 1988; 318:1638-44.
4. Pezella F, Tse AGD, Cordell JL, Pulford KAF, Gatter KC, Mason DY. Expression of the bcl-2 protein is not specific for the 14;18 chromosomal translocation. Am J Pathol 1990; 137:225-32.
5. Hockenberry DM, Zutter M, Hickey W, Nahm M, Korsmeyer S. bcl-2 protein is topographically restrict-

ed in tissues by apoptotic cell death. Proc Natl Acad Sci USA 1991; 88:6961-5.
6. Pezella F, Gatter K. What is the value of bcl-2 protein detection for the histopathologist? Histopatology 1995; 26:89-93.
7. Koersmeyer SJ. Bcl-2 initiates a new category of oncogenes: regulators of cell death. Blood 1992; 80:879-86.
8. McDonell TJ, Korsmeyer SJ. Progression from lymphoid hyperplasia to high grade malignant lymphoma in mice transgenic for the t(14;18). Nature 1991; 349: 254-6.
9. Linette GP, Hess JL, Sentman CL, Korsmeyer SJ. Diffuse malignant T cell lymphoma in lck-bcl-2- trasgenic mice. Blood 1995; 86:1255-60.
10. Yang E, Korsmeyer J. Molecular thanaptosis: a discourse on the bcl-2 family and cell death. Blood 1996; 88:386-401.
11. Merup M, Spasokoukotskaja T, Einhorn S, Smith E, Garthon G, Juliusson G. Bcl-2 rearrangements with breakpoints in both vcr and mbr in non-Hogkin's lymphoma and chronic lymphocytic leukaemia. Br J Haematol 1996; 92:647-52.
12. Nuñez G, London Y, Hockenberry D, Alexander M, Mckearn JP, Korsmeyer SJ. Deregulated bcl-2 gene expression selectively prolongs survival of growth factor-deprived hemopoietic cell lines. J Immunol 1990; 144:3602-10.
13. Campos L, Roualt JP, Sabido O, et al. High expression of bcl-2 protein in acute myeloid leukemia cells is associated with poor response to chemotherapy. Blood 1993; 81:3091-6.
14. Porwit-McDonald A, Ivory K, Wilkinson S, Wheatley K, Wong L, Janossy G. bcl-2 protein expression in normal bone marrow precursors and in acute leukemia. Leukemia 1995; 9:1191-8.
15. Coustan-Smith E, Kitanaka A, Pui CH, et al. Clinical relevance of bcl-2 overexpression in childhood acute lymphoblastic leukemia. Blood 1996; 87:1140-6.
16. Maung ZT, McLean M, Reid MM, et al. The relationship between bcl-2 expression and response to chemotherapy in acute leukaemia. Br J Haematol 1994; 8:105-9.
17. Zutter M, Hockenbery D, Silverman G, Korsmeyer J. Immunolocalization of the bcl-2 protein within hematopoietic neoplasms. Blood 1991; 78:1062-88.
18. Pezella F, Jones M, Ralfkier E, Ersboll J, Gatter KC, Mason DY. Evaluation of bcl-2 protein expression and 14;18 traslocation as prognostic markers in follicular lymphoma. Br J Cancer 1992; 65:87-92.
19. Piris MA, Pezella F, Martinez-Montero JC, et al. p53 and bcl-2 expression in high grade B-cell lymphomas: correlation with survival time. Br J Cancer 1994; 69:337-41.
20. Hermine O, Haioun C, Lepage E, et al. Prognostic significance of bcl-2 protein expression in aggressive non-Hodgkin's lymphoma. Blood 1996; 87:265-72.
21. Hill M, Kenneth A, McLenan A, et al. Prognostic significance of bcl-2 expression and bcl-2 major breakpoint region rearrangement in diffuse large cell non-Hodgking lymphoma: a British National Lymphoma Investigation study. Blood 1996; 88:1046-51.
22. Schena M, Larsson LG, Gottardi D, et al. Growth and differentiation associated expression of bcl-2 in B chronic lymphocytic leukemia cells. Blood 1992; 79:2981-9.
23. Miyashita T, Reed JC. Bcl-2 oncoprotein blocks chemotherapy induced apoptosis in a human leukemia cell line. Blood 1993; 81:151-7
24. Pettersson M, Jernberg-Wicklund H, Larsson LG, et al. Expression of the bcl-2 gene in human multiple myeloma cell lines and normal plasma cells. Blood 1992; 79:495-502.
25. Hamilton MS, Barker HF, Ball J, Drew M, Abbot SD, Franklin IM. Normal and neoplastic plasma cells express the bcl-2 antigen. Leukemia 1991; 5:768-71.
26. Durie BGM, Mason DY, Giles F, et al. Expression of the bcl-2 oncogene protein in multiple myeloma. [abstract]. Blood 1990; 76 (Suppl 1):374a.
27. Tu Y, Xu FH, Liu J, Vescio R, Berenson J, Fady C, Lichtenstein A. Upregulated expression of bcl-2 in multiple myeloma cells induced by exposure to doxorubicin, etoposide and hydrogen peroxide. Blood 1996; 88: 1805-12.
28. Kyle RA, Lust JA. Monoclonal gammopathy of undeterminated significance. Semin Hematol 1989; 26: 176-200.
29. Chronic Leukemia-Myeloma Task Force, National Cancer Institute proposed guidelines for protocol studies. II. Plasma cell myeloma. Cancer Chemother Rep 1973; 4:145-58.
30. Durie BGM, Salmon SE. A clinical staging system for multiple myeloma: correlation of measured myeloma cell mass with presenting clinical features, response to treatment and survival. Cancer 1975; 36:842-54.
31. Cordell JL, Fallini B, Erber WH, et al. Immunoenzimatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal antialkaline phosphatase (APAAP complexes). J Histochem Cytochem 1984; 32:219-22.
32. Mason DY, Cordell JL, Abdullazid Z., Natem M, Bordanare G. Preparation of peroxidase-antiperoxidase (PAP) complexes for immunohistological labelling of monoclonal antibodies. J Histochem Cytochem 1982; 30:1114-22.
33. Miguel-García A, Matutes E, Tarín F, et al. Circulating Ki-67 positive lymphocytes in multiple myeloma and bening monoclonal gammopathy. J Clin Pathol 1995; 48:835-9.
34. Kaplan EL, Meier P. Non-parametric estimation from incomplete observations. JAMA 1958; 53:475-81.
35. Mantel N. Evaluation of survival data and two new rank order statistics arising in its considerations. Cancer Chemoter Rep 1966; 50:163-70.
36. Schena M, Larsson LG, Gottardi D, et al. Growth- and differentiation-associated expression of bcl-2 in B-chronic lymphocytic leukemia cells. Blood 1992; 11: 2981-9.
37. Wilson W, Teruya-Feldstein J, Fest T, et al. Relationship of p-53, bcl-2 and tumor proliferation to clinical drug resistance in non-Hodgkin's lymphomas. Blood 1997; 89:601-9.
38. Wolowiec D, Mekki Y, Ffrench P, et al. Differential expression of cell proliferation regulatory proteins in B- and T-lineage acute lymphoblastic leukaemias. Br J Haematol 1996; 95:518-23.
39. Winter JN, Andersen J, Reed JC, et al. bcl-2 expression correlates with lower proliferative activity in non-Hodking's lymphoma (NHL), an ECOG study [abstract]. Blood 1995; 86(Suppl 1):1351 a.
40. Kramer MHH, Hermans J, Parker J, et al. Clinical significance of bcl-2 and p-53 protein expression in diffuse large B cell lymphoma: a population based study. J Clin Oncol 1996; 14:2131-8.
41. Pepper C, Bentley P, Hoy T. Regulation of clinical chemoresistance by bcl-2 and bax oncoproteins in B cell chronic lymphocytic leukaemia. Br J Haematol 1996; 95:513-7.
42. Ong F, Nieuwkoop JA, Groot-Swings GMJS, et al. Bcl-2 protein expression is not related to short survival in multiple myeloma. Leukemia 1995; 9:1282-4.

Ferrata Storti Foundation