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PLASMA CELL P170 EXPRESSION AND RESPONSE TO TREATMENT IN MULTIPLE MYELOMA

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ABSTRACT

Background. **Vincristine and anthracyclines are first-line agents for the treatment of multiple myeloma (MM). P170-related multidrug resistance (MDR) may influence the response to these drugs.**

Materials and Methods. **P170 expression of bone marrow plasma cells was assayed by immunocytochemistry (alkaline phosphatase anti-alkaline phosphatase technique) with the MRK-16 monoclonal antibody. A case was considered positive if one per cent or more of plasma cells stained as strongly as positive controls.**

Results. **Six of 17 (35%) cases in relapse and 18/72 (25%) at diagnosis were MDR positive. MDR positivity was not found in micromolecular MM and was significantly associated with the serum β2-microglobulin level. Response to treatments including dexamethasone, vincristine and doxorubicin, or idarubicin, or mitoxantrone was independent of MDR positivity (50% in positive cases vs. 56% in negative ones).**

Conclusions. **The detection of P170 in bone marrow plasma cells with the currently available methodology is not likely to predict response to treatments that include vincristine, anthracyclines or mitoxantrone. Further studies are required to evaluate the relevance of P170-related MDR to the development of MM therapy.**

Key words: multidrug resistance, P170 glycoprotein, multiple myeloma, anthracycline

Resistance to cytotoxic agents prevents tumor eradication or reduction in size to a level that would be consistent with a long and stable complete remission. Therefore drug resistance is the main cause of treatment failure.

Multiple myeloma (MM) is an example of a tumor in which the response to chemotherapy is usually partial and is followed by a progression that is more and more resistant to treatment, so that all these patients eventually die of the myeloma or of treatment-related complications. Drug resistance is clearly multifactorial and several different mechanisms are likely to be operative at the same time.[1-2]

A well-known factor in pleiotropic or non-specific multidrug resistance (MDR) was identified in a 170 Kd transmembrane glycoprotein (P170 or Pgp) encoded by the mdr-1 gene in chromosome 7.[3,4]

P170 works as an efflux pump that prevents intracellular accumulation of a broad spectrum of structurally and functionally unrelated natural compounds, including anthracyclines, anthracenedione and epipodophylline derivatives, and vinca alkaloids.[5,6] Because these drugs are currently employed in first-line treatment of MM,[7-8] we investigated P170 expression in MM plasma cells and the relationship between P170 and response to treatment.

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Materials and Methods

Patients

This study included 72 consecutive MM patients who were seen and studied prior to any treatment between May 1990 and February 1995. Diagnosis was made according to the *Chronic Leukemia-Myeloma Task Force* guidelines[9] and staging was based on Durie and Salmon criteria.[10] Thirty-five patients were male and 37 were female. Median age was 60 (range 40 to 79) years. Twenty-seven patients (37%) were in stage I, 7 patients (10%) in stage II and 38 patients (53%) were in stage III. The M component was IgG in 46 cases (64%) and IgA in 15 (21%), while 11 patients (15%) had a micromolecular myeloma. In 9 patients (12%) the serum creatinine concentration was greater than 20 mg/L (stage B). The β2 microglobulin level was evaluated in 68 patients and was found to be more than 6 mg/L in 10 cases (15%). In addition to the 72 patients described above, 17 others were studied after first-line treatment in first or subsequent relapse. The data from these patients were utilized only comparison purposes between P170 expression and treatment results.

Treatment

The relationship between P170 expression and response to treatment was examined in 38 patients who were first treated with a combination of MDR-related drugs, namely vincristine, doxorubicin, mitoxantrone or idarubicin, and in 17 other patients who received the same treatment at relapse or progression of MM. Therefore the total number of cases that could be evaluated for this relationship was 55. Treatment consisted of VAD [vincristine 0.4 mg/24 h by continuous i.v. infusion, doxorubicin (adriamycin) 9 mg/m^2/24 h by continuous i.v. infusion, and dexamethasone 40 mg i.v. daily for 4 days] in 31 cases. In 17 others doxorubicin was substituted by mitoxantrone 3 mg/m^2/24 h (VMD course), and in the remaining 7 cases doxorubicin was substituted by idarubicin 3 mg/m^2/24 h (VID course). All patients were given at least 4 courses of chemotherapy. Response to treatment was defined by a reduction in the serum M component or in light chain proteinuria to less than 50% of the pretreatment value for at least three consecutive months after the 4th course of chemotherapy. Disease progression was defined by an increase in the serum M component or in light chain proteinuria of more than 25% of the pretreatment value or by any radiological, laboratory or clinical evidence of progression. The cases that did not fit the criteria for response or for disease progression were classified as stable disease.

Methods

Marrow was aspirated from the posterior iliac spine. Samples were anticoagulated with heparin and cells were separated on Ficoll-Hypaque, harvested, washed twice in 0.05 M Tris-buffered solution (TBS) (pH 7.4), and resuspended in TBS at a final concentration of 1×10^6 cells/mL. Cytospin preparations were air dried for 24 hours at room temperature and processed for the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique, as described elsewhere.[11,12] All samples were studied with the P170-directed monoclonal antibody MRK-16 as the primary antibody. MRK-16 is an IgG2 that reacts with a P170 epitope on the external surface membrane of the cells.[13] Cytospins were exposed to irrelevant isotype-matched antibodies as negative controls. Positive controls were provided by a MDR$^+$ colon adenocarcinoma cell line (LOVO DX), which was grown in the presence of doxorubicin (100 ng/mL).[11] Plasma cells were identified morphologically and scored by comparison with the positive LOVO-DX control as strongly positive, moderately positive, weakly positive or negative. Scoring was performed blindly and separately by three observers. All three observers agreed that all samples contained a variable proportion of positive cells but concluded that the distinction between moderately positive and weakly positive cells was actually unreliable, not only for the differences between one observer and another but also because different data could be provided by the same observer in subsequent blind observations. On the other hand, all three observers were able to agree on the presence of strongly positive cells (Figure 1). Therefore, for the purposes of this study, cases were scored as

negative or positive according to the absence or presence of strongly positive plasma cells.

Results

Seventeen patients were studied at the time of relapse or progression. Six of them (35%) were classified as MDR positive. In these cases the percentage of strongly positive plasma cells ranged between 2% and 80%. Seventy-two patients were studied at diagnosis and prior to any treatment. Eighteen of them (25%) were classified as MDR positive. In these cases the percentage of strongly positive plasma cells ranged between 1% and 90% (median 30%). In the 72 cases who were studied prior to any treatment, the relationship between MDR positivity and disease features was analyzed, and the main results are shown in Table 1. MDR positivity was unrelated to the stage of the disease or to the serum creatinine level, but it was related to the M-protein isotype because P170 overexpression was most frequent in IgG MM (16 of 46 or 35%), was rare in IgA MM (2 of 15 or 13%), and was not found in any of the 11 cases with the micromolecular type. Sixty per cent of the cases with a high serum β2-microglobulin level (more than 6 mg/L) were positive, as compared with 19% of the other 58 cases ($p = 0.018$). The rate of response to the regime with dexamethasone, vincristine and adriamycin (VAD) or mitoxantrone (VMD) or idarubicin (VID) was 71% in the 38 patients who were treated soon after diagnosis, and only 18% in the 17 patients who were treated in relapse or in progression. However, the response rate was independent of MDR positivity or negativity (Table 2) and of the compound that was used (Table 3). Survival was the same in MDR-positive and in MDR-negative cases (median 27.5 months vs. 34 months, $p = 0.93$).

Discussion

The expression of the mdr-1 gene in MM has mainly been investigated by immunocytochemical assays with different P170-directed monoclonal antibodies (Table 4). Among the patients who were studied prior to any treatment, the

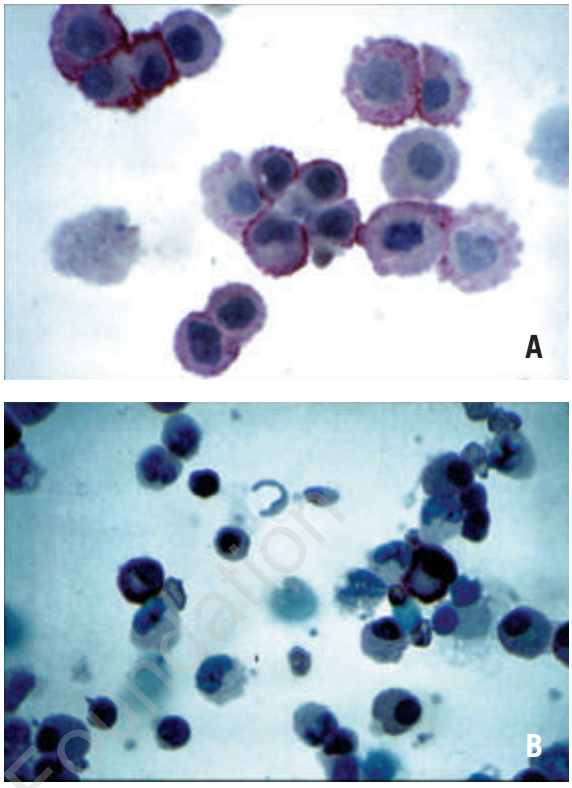

Figure 1. Examples of cell reactivity to MRK-16 by immunocytochemistry (APAAP). A) The positive control (LOVO DX cells); B) A positive case with two strongly positive plasma cells.

Table 1. Case distribution of P170 overexpression (MDR+) in the 72 patients who were studied prior to any treatment. Chi-square test, p-values are two sided.

Stage			
I	6/27	(22%)	
II	3/ 7	(43%)	P = 0.512
III	9/38	(24%)	
M-component			
IgG	16/46	(35%)	
IgA	2/15	(13%)	P = 0.209
BJ	0/11	//	
Serum creatinine			
< 20 mg/L	14/63	(22%)	
≥ 20 mg/L	4/ 9	(44%)	P = 0.303
Serum β_2-microglobulin			
< 6 mg/L	11/58	(19%)	
≥ 6 mg/L	6/10	(60%)	P = 0.018

percentage of those defined as positive ranged from 0[14] to 41%[15] vs. 25% in our study. Among the patients who were studied in relapse or in progression and had received prior treatment, the percentage of *positive* cases ranged between 29% and 59% vs. 35% in our study. Using flow cytometry and MRK-16, Solary *et al.*[16] found a positivity in 5 of 16 cases (31%). Employing slot blot, Linsenmeyer *et al.*[17] found an increase of mdr-1 mRNA in 18 of 36 cases (50%) studied at diagnosis and in 8 of 14 (57%) studied in relapse. It is noteworthy that the criteria used to define the positivity of a plasma cell and to identify a case as positive were different in all the studies.

The results of the present study confirmed that in some cases of MM a variable proportion of plasma cells express or overexpress P170, but they did not confirm the suggestions that have been made regarding the negative relationship between P170 expression and response to treatment.[18] These suggestions were based on early reports[15,17,19-25] and were reinforced by the finding that P170 expression was related to the extent of prior exposure to vincristine and doxorubicin.[26] Moreover, Sonneveld *et al.*[27,28] observed that cyclosporine administration *in vivo* was able to restore sensitivity to VAD in patients who were resistant to it. However, two studies on larger series of 60 and 123 cases that were refractory to alkylating agents failed to show a relation between P170 expression and response to VAD,[29,30] and failed to show a benefit from the addition of verapamil to VAD.[30]

Nevertheless, it should not be overlooked that several studies provided evidence that P170-positive malignant plasma cells are not able to accumulate doxorubicin, are more resistant to this compound and can become more sensitive to doxorubicin upon exposure to verapamil or

Table 2. Case distribution according to response to treatment and MDR positivity in the 38 patients who were first treated with VAD, VMD or VID at onset, and in the 17 patients who received the same treatment at the time of progression or relapse.

	Treated at onset	*Treated at progression*	*Total*
No. response/No. total	27/38 (71%)	3/17 (18%)	30/55 (54%)
MDR POSITIVE No. response/No. total	7/10 (70%)	1/6 (17%)	8/16 (50%)
MDR NEGATIVE No. response/No. total	20/28 (71%)	2/11 (18%)	22/39 (56%)

Table 3. Case distribution according to response to treatment and MDR positivity with relationship with the treatment regime, that contained either doxorubicin (adriamycin) (VAD), or mitoxantrone (VMD) or idarubicin (VID).

	VAD	*VMD*	*VID*	*TOTAL*
No. response/No. total	16/31 (52%)	11/17 (65%)	3/7 (43%)	30/55 (54%)
MDR positive, No. response/ No. total	5/9 (55%)	3/5 (60%)	0/2 //	8/16 (50%)
MDR negative, No. response/No. total	11/22 (50%)	8/12 (67%)	3/5 (60%)	22/39 (56%)

Table 4. Frequency of MDR-positive cases in this study and in 6 others in which P170 expression was assayed by immunocytochemistry (APAAP or immunoperoxidase) with a P170-directed monoclonal antibody (C-219, JSB-1, or MRK-16). The definition of positivity was different in all the studies.

References	*Reagents and methods*	*No prior treatment*	*Prior treatment*
Riccardi et al, 1991	C-219, APAAP	18/44 (41%)	–
Sonnenveld et al, 1992	C-219/JSB-1, APAAP	-	12/21 (57%)
Grogan et al, 1993	JSB-1, PEROX	3/47 (6%)	21/49 (43%)
Ishikawa et al, 1993	MRK-16, PEROX	0/25 (–)	–
Cornelissen et al, 1994	C-219, APAAP	–	37/63 (59%)
Dalton et al, 1995	C-219/JSB-1, PEROX	–	12/41 (29%)
This study	MRK-16, APAAP	18/72 (25%)	6/17 (35%)

other MDR modifiers.[16,19,20,31,32] It is also important to notice that analysis of the relationship between mdr-1 gene expression and response to treatment is complicated by the fact that all treatments have also included corticosteroids, which are not processed by P170, and that other P170-unrelated mechanisms of multidrug resistance may be operative.[1,2,14,17] Moreover, methods are not yet standardized and it is currently impossible to establish what amount of gene expression may be clinically relevant at the single-cell level and what proportion of positive cells is required to identify a case as MDR positive.[18,33,34] It should also not be overlooked that a tumor with 10^{12} cells and one in a thousand plasma cells positive would hardly be defined as a positive neoplasm, but it would actually contain a substantial number of MDR plasma cells, i.e. 10^{9}.

In conclusion, there are still many good reasons for careful evaluation of P170-related MDR in MM including the recent report by Pilarski *et al.*,[32] who found that in the peripheral blood of MM B-cell progenitors of malignant plasma cells overexpress P170 and are resistant to doxorubicin. The proliferative potential of these cells may be relevant to the emergence of resistance over the time. However, the appreciation of P170 in marrow plasma cells by the current monoclonal antibody-based methodology is not likely to help in patient care and management.

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