Multiple Myeloma: Increased Circulating Lymphocytes Carrying Plasma Cell-Associated Antigens as an Indicator of Poor Survival

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In multiple myeloma (MM) an increase in circulating lymphocytes expressing plasma cell-associated antigens (PCAA) has been described. Its prognostic significance was evaluated in this study. The immunologic phenotype of peripheral blood lymphocytes was analyzed with a panel of monoclonal antibodies specific for B, T, natural killer lymphocytes, and PCAA (CD38, PCA1) in 52 MM patients at diagnosis, remission, and during relapse, 18 monoclonal gammopathy of undetermined significance (MGUS), and 25 normal controls. No significant phenotypic alteration was observed in MGUS. In MM, the number of B lymphocytes was in the normal range at diagnosis and during the subsequent phases. A CD4/CD8 ratio decrease, during relapse, was due to both a $CD4^+$ reduction and to an expansion of a subset of $CD8^+$ activated suppressor lymphocytes. $CD38^+$ and $PCA1^+$ lymphocytes at diagnosis were significantly higher than in MGUS, and a further increase was observed during relapse, suggesting a correlation between PCAA expression and disease activity. The prognostic significance of increased PCAA was confirmed by a survival analysis of 32 patients evaluated at diagnosis using a CD38 cutoff of 0.45 × 10^9/L positive lymphocytes. Median survival for patients with high values was only 14 months, whereas it was not reached at 32 months by those with low values ($P < .0007$).

IN MULTIPLE MYELOMA (MM), phenotypic and functional alterations of several lymphocyte subsets have been described. Anti-idiotypic antibodies specific for each patient have been used to detect circulating tumor cells, and they seem to represent the entire spectrum of the B-cell differentiation pathway in peripheral blood.[1-6] Kubagawa et al[2] has identified circulating pre-B lymphocytes belonging to the tumor clone, and King and Nelson[3] have described tumor cells coexpressing lymphocyte and plasma cell antigens. Molecular analysis has corroborated these findings: lymphocytes with the same immunoglobulin (Ig) gene rearrangement as bone marrow myeloma cells have been reported.[5,6] Moreover, a correlation between clinical progression and the number of circulating neoplastic cells has been suggested.[1]

Phenotypic alterations in T-cell subsets, such as a decreased CD4/CD8 ratio[7,8] and an increased number of activated T cells, expressing the CD74 (HLA-DR) have been also described.[9] T cells seem unable to mediate normal cytotoxic and LAK activities.[10,11] Moreover, these alterations are more evident in patients with active MM.

A significant increase in circulating lymphocytes expressing plasma cell-associated antigens (PCAA), such as CD38 or PCA1, has also been reported.[12,13] It is well-known that these antigens are expressed by both terminally differentiated B lymphocytes and other hematopoietic cells, such as activated T lymphocytes.[14] A two-color analysis has illustrated the expression of PCAA by B and T cells.[12] This increase of both B neoplastic cells and activated T cells is considered a marker of poor prognosis.[1,9] In a preliminary study, both of these cell subsets, detected by the analysis of PCAA expression, were significantly higher in MM than in monoclonal gammopathy of undetermined significance (MGUS), and in MM in remission phase than in relapse phase.[12]

In the present study the immunologic phenotype of 52 MM patients in different phases of the disease and 18 MGUS was evaluated. Moreover, a survival analysis was performed to assess the prognostic significance of the expression of PCAA by circulating lymphocytes.

MATERIALS AND METHODS

Patients. Seventy patients with monoclonal gammopathies entered this study from February 1986 to June 1989. Eighteen had MGUS and 52 MM (33 men, 19 women, aged 58.5 ± 8.9 years). MGUS showed a stable M component for at least 2 years and a bone marrow labeling index less than 1%.[15] MM was diagnosed according to the Southwest Oncology Group (SWOG) criteria.[16] Remission was defined as an M component decrease greater than 50% lasting for at least 6 months without treatment (unmaintained remission phase),[17] and relapse as an increase greater than 50% from the lowest value. According to the Durie and Salmon staging system,[18] 5 were stage I, 20 stage II, and 27 stage III; 6 were sub-stage B. Twenty-six patients were IgG, 17 IgA, 8 Bence Jones myeloma, and 1 IgD. Thirty-two MM patients were evaluated at diagnosis. Median follow-up for censored patients was 13 months (range 1 to 38).

Patients were treated with 12 courses of melphalan and prednisone or alternating VMCP/VBAP: in a previous report we showed that these regimens were equally effective even in high-risk patients.[19]

The 25 normal controls were healthy subjects comparable for mean age and sex distribution with our patient population, and screened for platelet aggregation.

Phenotypic analysis. A total of 135 phenotypic analyses of peripheral blood lymphocytes (PBL) were performed. In the 32 MM patients evaluated at diagnosis the analysis was also performed in remission and relapse (Table 1).

PBL were separated on Lymphoprep (Nyegaard, Oslo, Norway) gradient, and monocytes were removed by adherence on plastic culture flasks (Falcon, Becton Dickinson, Mountain View, CA) at 37°C for 60 minutes. In double-layer staining, lymphocytes were

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Table 1. Number of Phenotypic Analysis Performed in Different Patient Subgroups

			Phenotypic Analysis
MM	52	Diagnosis	32
		First remission	20
		Relapse	40
MGUS	18		18
Controls	25		25
Totals	95		135

incubated for 30 minutes at 4°C with 5 μL of monoclonal antibody (MoAb), washed twice, and then incubated for 30 minutes with 2.5 μL of FITC-conjugated goat antimouse Igs. At least 5,000 cells were scored using a Facscan (Becton Dickinson).

B1 (CD20), B4 (CD19), and PCA1 were purchased from Coulter Clone (Luton, England); Leu 4 (CD3), Leu3 (CD4), Leu2 (CD8), transferrin receptor (CD71), CALLA (CD10), Leu15 (CD11b), HLA-DR (CD74), LEU7 (CD57), and fluorescein-conjugated goat antimouse Ig from Becton Dickinson; and HAN-PC1 (CD38) was kindly provided by Dr K. Thielemans (Vrije Universiteit Brussel, Brussels, Belgium).

Statistical analysis. Results are expressed as absolute number per liter. Quantile-quantile plots were used to assess the normality of the data.[20] Because the majority of data sets were not normally distributed, data were summarized as median (range). The Kruskal-Wallis test was used[21] to simultaneously compare the expression of each lymphocyte antigen analyzed in five subgroups: controls, MGUS, MM at diagnosis, remission, and relapse. If statistically significant, comparisons between pairs of interest were performed with the Wilcoxon test. The pairs of interest were: normal control versus MGUS, MGUS versus MM at diagnosis, MM at diagnosis versus MM at relapse, and MM at remission versus MM at relapse. Because of the large number of tests run ($4 \times 13 = 52$), the increased risk of significant results obtained just by chance was tapered by Bonferroni's correction.[22] Therefore, the significance level of P value was set to .001. The Kaplan-Meier method was used to estimate survival, and the log-rank test to compare survivals.[23,24] Correlation analysis was performed using Pearson's test. All data were processed with the SAS statistical software package (SAS Institute Inc, Cary, NC).

RESULTS

PBL immunologic phenotypes of the 52 MM, 18 MGUS patients, and the 25 controls are shown in Table 2.

In MGUS patients no phenotypic alteration was detected. In MM patients, no significant phenotypic variation was present in the B-cell compartment (CD19, CD20) both at diagnosis or during remission and relapse. The number of T cells detected by the expression of CD3 was in the normal range, whereas the CD4/CD8 ratio decreased during the course of the disease due to both a progressive increase in $CD8^+$ and a constant low value in $CD4^+$ lymphocytes. These data, together with the high expression of $CD11b^+$ and $CD74^+$ lymphocytes, point to the elevation of activated suppressor cells, as previously demonstrated.[9] The transferrin receptor (CD71), another activation marker, was also higher in MM at diagnosis than in MGUS and normal controls, normal during remission, and high during relapse. $CD10^+$ lymphocytes sharply increased during relapse in four patients only, suggesting an intrinsic individual patient characteristic rather than a general feature. The natural killer subset, identified by the CD57, showed no significant variation at diagnosis and during follow-up.

In the 32 MM patients evaluated at diagnosis, a significant increase of $CD38^+$ lymphocytes was detected in comparison with MGUS (Table 2). Among MM patients, $CD38^+$ lymphocytes were significantly increased during relapse. Similar variations were observed for PCA1 expression, but they did not reach the statistical significance using Bonferroni's correction (Table 2). A statistically significant correlation was detected between the number of $CD38^+$ and $PCA1^+$ lymphocytes ($r = .71$; $P < .0001$), suggesting their partial coexpression by the same cell; this was confirmed by a two-color analysis in five patients (data not shown). Figure 1 shows the distribution of $CD38^+$ lymphocytes in normal controls, in MGUS, and in MM during different phases of the disease.

To assess the prognostic value of PCAA expression, a survival analysis was performed in 32 MM patients evalu-

Table 2. Immunologic Phenotype of PBL in Normal Controls, MGUS, and MM Patients

Groups N. Phenotypic Analysis	Normal Controls 25	MGUS 18	MM Diagnosis 32	MM Remission 20	MM Relapse 40	P
CD20	0.24 (0.04-0.36)	0.13 (0.05-0.37)	0.16 (0.05-0.80)	0.17 (0.03-0.41)	0.20 (0.01-0.75)	NS
CD19	0.30 (0.04-0.42)	0.17 (0.01-0.47)	0.21 (0.06-0.79)	0.17 (0.04-0.49)	0.22 (0.01-0.75)	NS
CD74	0.20 (0.08-0.36)	0.19 (0.04-0.73)*	0.31 (0.08-0.61)*	0.40 (0.04-1.16)	0.34 (0.21-1.00)	.0005
CD10	0.04 (0.00-0.28)	0.04 (0.00-0.31)	0.10 (0.00-0.57)	0.07 (0.01-0.24)	0.14 (0.01-1.10)	.0001
CD3	1.30 (0.60-1.60)	0.90 (0.45-1.87)	1.04 (0.62-1.74)	1.08 (0.34-1.64)	1.06 (0.42-1.69)	NS
CD4	0.86 (0.36-1.28)	0.81 (0.10-1.50)	0.58 (0.35-1.25)	0.43 (0.17-0.86)	0.53 (0.05-1.03)	.0001
CD8	0.48 (0.26-0.84)	0.44 (0.19-0.89)	0.39 (0.11-0.84)†	0.67 (0.10-0.94)	0.69 (0.01-1.62)†	.0003
CD4/CD8	1.70 (1.20-4.20)	1.10 (1.30-3.30)	1.60 (0.40-5.20)†	0.90 (0.30-3.00)	0.80 (0.10-3.00)†	.0001
CD71	0.08 (0.02-0.19)	0.09 (0.02-0.23)*	0.18 (0.02-0.27)*	0.09 (0.03-0.35)	0.18 (0.04-0.79)	.01
CD38	0.24 (0.04-0.48)	0.19 (0.01-0.44)†	0.38 (0.02-1.10)†	0.38 (0.07-0.92)‡	0.68 (0.06-1.13)‡	.0001
PCA-1	0.20 (0.14-0.32)	0.10 (0.01-0.37)*	0.21 (0.05-1.15)*	0.19 (0.05-0.47)§	0.39 (0.05-1.32)§	.0001
CD11b	0.19 (0.08-0.50)	0.10 (0.01-0.32)†	0.34 (0.05-0.89)†	0.35 (0.07-0.74)	0.39 (0.02-0.74)	.0003
CD57	0.24 (0.10-0.70)	0.36 (0.15-1.19)	0.35 (0.07-0.74)	0.41 (0.09-0.81)	0.48 (0.01-0.91)	NS

Values are expressed as absolute number of positive cells (median and range $\times 10^9$/L). Kruskal-Wallis P values are indicated in right column. According to Bonferroni's correction, Wilcoxon two-sample test is significant if $P < .001$. Wilcoxon two sample test: *$P < .01$; †$P < .0006$; ‡$P < .001$; §P .003.

Abbreviation: NS, not significant.

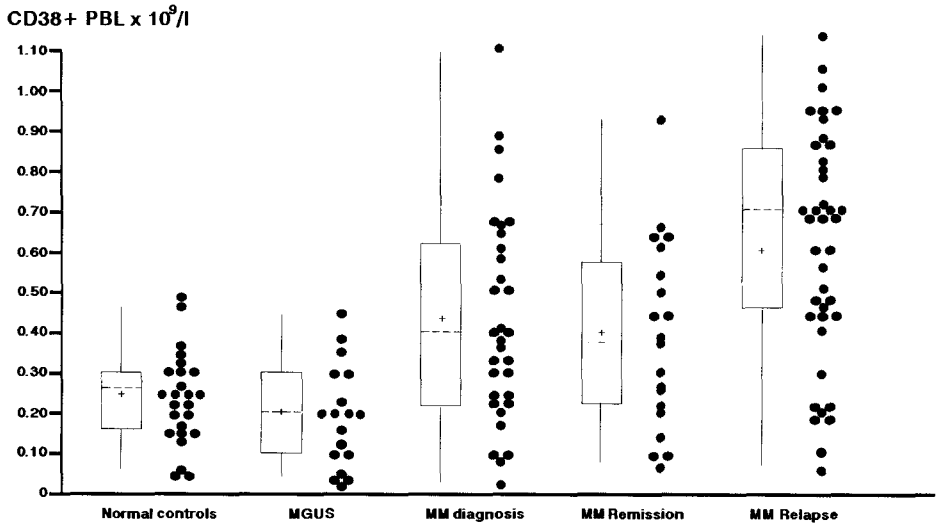

Fig 1. Distribution of CD38+ lymphocytes in normal controls, MGUS, and MM patients in different phases of the disease. Box plot legend: dashed horizontal line, median; plus sign, mean; box, middle 50% of values; vertical lines, up to 95% of distribution.

ated at diagnosis. In the CD38 analysis, a cutoff of 0.45 × 10^9/L was chosen because it gave the best patient separation. Clinical characteristics of the two groups of patients separated according to this CD38 cutoff are described in Table 3. Median survival of patients with CD38+ lymphocytes greater than 0.45 × 10^9/L was 14 months, whereas patients with lower values did not reach the median survival at 32 months ($P < .0007$) (Fig 2).

It may be stressed that all data are presented as the absolute number of circulating lymphocytes. Even more significant results were obtained on considering the percentage of positive lymphocytes, particularly in the case of CD38 and PCA1 expression. MM patients displayed a significant lymphocytopenia in comparison with normal controls (median 1,561 *v* 2,073; $P < .0001$). An increase in the percentage of lymphocytes carrying PCAA resulted in subset unbalance and was frequently not evident when absolute numbers were considered.

Table 3. Clinical Characteristics of MM Patients at Diagnosis

	CD38+ <0.45 × 10^9/L (19 pts)	CD38+ ≥0.45 × 10^9/L (13 pts)	*P*
Sex M	12	8	NS
F	7	5	
Stage I	2	2	
II	8	4	NS
III	9	7	
M-Comp IgG	10	4	
IgA	6	5	NS
IgD	0	1	
BJ	3	3	
Age	57.7 ± 9.9	57.4 ± 10	NS
LI%	1.0 ± 0.7	1.1 ± 0.9	NS

Abbreviations: NS, not significant; LI, labeling index.

DISCUSSION

Alterations of the immunologic phenotype of virtually all PBL subsets have been reported in MM.[7-10,12,13,25] This report shows that the number of circulating lymphocytes carrying PCAA[12,13] is related to the course of the disease: values were higher during relapse. Moreover, a survival analysis showed that the number of PCAA+ lymphocytes at diagnosis is a useful prognostic parameter because median survival for patients with a high number was significantly reduced.

The immunologic phenotype of 52 MM patients and 18 MGUS was evaluated with a panel of MoAbs. No significant variation in the B-lymphocyte compartment was shown by the B4 (CD19) and B1 (CD20) MoAbs. A reduction in CD4+ and an increase in CD8+ lymphocytes were particularly evident during relapse, leading to the previously described reduction of the CD4/CD8 ratio.[7,8] Investigation of

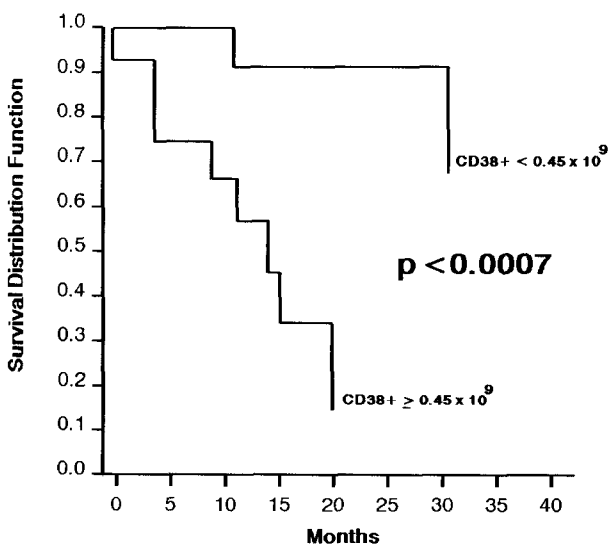

Fig 2. Survival curves of MM patients with CD38+ greater than 0.45 × 10^9/L (19 patients) or less than 0.45 × 10^9/L (13 patients). Curves are plotted from diagnosis.

the T-cell compartment confirmed the expansion of activated suppressor cells carrying CD8, CD11b, and CD74 (HLA-DR) antigens. Moreover, they coexpress PCAA, react against the related patient M component Ig idiotype,[9] and may be considered specifically directed against the tumor clone. The nature and possible function of these T cells is still undetermined. As a first hypothesis, they may be regarded as an attempt by the immune system to suppress the tumor clone when an expansion occurs. The transferrin receptor (CD71), another activation marker, was increased in MM at diagnosis, returned to normal values during remission phase, and increased again during relapse.

An increase in PCAA$^+$ PBL, unaccompanied by circulating plasma cells, has been previously reported.[12,13,26] These cells have been regarded as neoplastic precursor cells. Their malignant origin is suggested by immunologic studies using anti-idiotypic antibodies[1-4] and molecular analysis of the Ig genes showing the same rearrangement as that present in the bone marrow myeloma cells.[5,6] Moreover, a B clonal excess detected by the unbalance of the K/λ Ig light chain ratio has been described in several MM patients.[13] However, PCAA are also expressed by T-cell blasts, and we have already demonstrated that they significantly contribute to the increase of positive PCAA cells.[12]

Phenotypic analysis of PBL performed with PCAA takes into account the presence of neoplastic precursors and activated T cells, which both expand during relapse.[1,9] We now confirm the expansion of PCAA positive lymphocytes during this phase. In our patient series, PCAA values were highly dispersed at diagnosis, suggesting that they do not just increase during the course of the disease, but may already indicate a poor prognosis at diagnosis. Therefore, we performed a survival analysis according to the PCAA expression: median survival for patients with more than 0.45×10^9/L CD38$^+$ lymphocytes was only 14 months. This feature definitely confirms its prognostic significance. From a clinical point of view, a high CD38 value may be relevant to: (1) discriminate between MM and MGUS; (2) determine whether the disease is stable or progressing; or (3) evaluate the prognosis at diagnosis.

Because phenotypic analysis of PBL with MoAbs is a routine practice in several hematologic centers, it may be regarded as a simple alternative to more complex analyses requiring bone marrow samples, such as plasma cell labeling index,[19] in the evaluation of MM prognosis.

In conclusion, PCAA phenotypic analysis in combination with other prognostic factors, such as serum β-2 microglobulin,[27] can be used to select poor prognosis patients as potential candidates for the newly proposed aggressive chemotherapy regimens.[28]

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