Myeloma Bone Marrow Acid Phosphatase Staining: A Correlative Study of 38 Patients

By Regis Bataille, Brian G. M. Durie, Jacque Sany, and Sydney E. Salmon

Acid phosphatase (AP) activity of plasma cells was studied in 38 patients with multiple myeloma (MM). The average activity per cell was strong (mean score = 2.42; maximum score = 4) and the percentage of positive cells was greater than 90% in over 71% of patients. The average AP activity per cell was higher prior to treatment (3.06 ± 0.53) compared to relapse (2.48 ± 0.77) and remission (1.81 ± 1.02; p < 0.05 and p < 0.01, respectively). In correlation of AP activity with clinical features at the time of the study, the only significant difference was between kappa and lambda subtype. Patients with lambda MM had a higher average AP activity per cell in remission (2.71 ± 0.43) as opposed to kappa MM (1.17 ± 1.06, p < 0.05 for difference). AP activity was not significantly correlated with degree of bone involvement. However, activity seemed to be a good marker of disease activity.

It has been well documented that acid phosphatase (AP) is present both in normal plasma cells and myeloma cells. Myeloma cells usually have more marked activity than normal plasma cells or plasma cells from patients with nonmalignant monoclonal components. Consequently, it has been suggested that elevated AP might be useful in the diagnosis of multiple myeloma (MM). Other enzymes present in abnormal amounts in myeloma cells include β-glucuronidase and nonspecific esterase, which are increased, and ATPase, which is present in reduced amount. In addition, it is thought that these lysosomal enzymes present in increased amounts, such as acid phosphatase or β-glucuronidase, might be involved in the pathogenesis of myeloma bone lesions.

For these reasons, we have examined AP activity in 38 patients with MM. The goals of our study were: (1) to assess plasma cell AP activity in patients with MM, pretreatment, in remission, and in relapse; and (2) to evaluate the significance of such an activity by studying the relationship between AP activity and certain clinical features of MM, such as hypercalcemia and degree of bone involvement. We obtained evidence that AP activity is very high in patients with MM and correlates well with disease activity. Acid phosphatase would therefore appear to be a potentially useful tumor marker, which relates well to disease activity and response to treatment.

MATERIALS AND METHODS

Thirty-eight patients with documented MM (with primary bone involvement) were included in the study. Clinical and immunologic criteria and clinical staging of MM have been previously described. There was a slight male preponderance (sex ratio M/F, 1.36) and the mean age was 61 ± 13 yr. Fifty-one percent (51%) had IgG myeloma, 31% IgA, and 18% Bence Jones only myeloma. Fifty-seven percent (57%) had kappa subtype and 43% lambda light-chain subtype. Twenty-five percent (25%) were either stage I or stage II, while 75% were stage III. Seventy-three percent (73%) of our patients had scoliosis. Hypercalcemia was noted in 3 patients at the time of the study. Seven patients had hypercalcemia prior to and one subsequent to the study.

Patients were tested during initial staging and at various intervals thereafter. The induction treatment phase was defined as at least 4 courses of pulse chemotherapy. Remission was defined as ≥50% reduction in the total body myeloma cell number or M-component production rate and relapse as at least a 50% increase in myeloma cell mass over the remission level. Twenty-two percent (22%) of patients were studied during induction, 26.7% during remission, and 51.3% while in relapse.

Patients were treated every 3–4 wk with intermittent 4-day pulse courses of combinations of cycle-nonspecific drugs (melphalan, cyclophosphamide, BCNU, adriamycin) plus prednisone with or without vincristine.

Bone marrow samples for AP activity were obtained 3–4 wk after the previous course of chemotherapy or prior to any treatment in the new patients. Bone marrow samples were sedimented with 3% dextran, and cytotoxicified smears were made as previously described. The cytochemical technique used in this study involved naphthol-AS-BI phosphoric acid as a substrate and pararosaniline as a coupler. This technique detects AP isoenzymes 1, 3, and 5. Under these conditions, the presence of AP activity was indicated by red granulations and/or diffuse cytoplasmic staining.

Ap scoring was as previously described, i.e., 0, negative reaction; 1, light red cytoplasmic shade; 2, less than 10 granulations; 3, more than 10 visible granulations, without confluence; 4, more than 10 granulations with confluence. Counts were made on 100 cells (except in a few patients). A score of AP activity per cell and a percentage of positive cells were calculated.

For statistical analysis, we used the Student’s t test and the chi-square method with or without Yates correction as necessary.

RESULTS

The average AP score per cell for all patients was 2.42. Fifty-four percent (54%) of patients had clearly high AP activity (≥2.50/cell), whereas 22% had weak
activity (<1.50) similar to normal plasma cells and plasma cells of patients with nonmalignant monoclonal components.3 The other 24% of patients had intermediate activity (>1.5 and <2.50). The percentage of positive cells was high: 100% in 45% and >90% in 71% of patients. In only 20% was the percentage of positive cells <80%. AP activity of >50% occurred in <5% of patients. The AP was usually identified near the Golgi apparatus.

The relationship of AP score to the clinical features of the disease (at the time of staining) was evaluated for the whole population. Parameters examined were age, immunoglobulin type, light-chain subtype, hemoglobin, serum calcium level, monoclonal component (serum and/or urine) levels, degree of bone involvement on radiographs, and exact stage as previously described.14 There were no relationships between the major clinical features or disease stage and AP activity. Of particular interest, there was no correlation between presence or absence of hypercalcemia nor extent of bone involvement and AP score. Conversely, as illustrated in Table 1, on review of AP score with respect to phase of disease and disease activity significant differences were found. Particularly noteworthy was the high AP activity per cell prior to induction therapy (3.06 ± 0.53), which was significantly higher than in remission (1.81 ± 1.02; p < 0.05). Moreover, there was a trend towards a lower average percentage of positive cells in remission (75.55% ± 8.69%) as compared to the preinduction phase (95.67% ± 9.78%; p < 0.1) as well as relapse (93.38% ± 11.93%; p < 0.05 for difference). An average AP score of greater than 3/cell was observed in 55% of patients in induction phase, but in 0% of patients in remission (p < 0.02).

As shown in Table 2, an especially interesting additional finding was the difference in AP score between patients with kappa and lambda light-chain subtype. In kappa patients, AP score was significantly lower in remission as compared to preinduction and at relapse (1.17 ± 1.06 compared to 3.23 ± 0.47 and 2.30 ± 0.79, respectively; p < 0.05). In contrast, for lambda patients, AP score did not fall in remission. For patients in remission AP score was significantly higher in the lambda patients compared to kappa (2.71 ± 0.43 compared to 1.17 ± 1.06; p < 0.05).

DISCUSSION

Acid phosphatase (AP) activity has been studied in normal plasma cells,1,2 plasma cells of patients with reactive plasmacytosis,7 or benign monoclonal components1 and myeloma cells.1-7 In particular, three recent studies have provided sufficient data for statistical analysis (Loffler, 1967; Hayhoe, 1976; Cassuto, 1977). These authors have all reported >90% positive cells and a high average AP activity per cell (>2.50) in MM as opposed to other plasma cell disorders (normal plasma cells, reactive plasmacytosis, and benign monoclonal components). Such data have stimulated interest in AP activity as a tumor marker for myeloma cells. Our present work confirms these results, there being more than 90% AP activity positive cells in 71% of our patients and a strong average activity per cell (3.06) prior to treatment. Perhaps more interesting was the low AP activity in some patients with MM: 22% of patients in our study compared with 10% and 20% of patients in prior reports (Loffler; Cassuto). This low AP activity is similar to that found in normal plasma cells.

One goal of our present study was to assess whether

### Table 1. Plasma Cell Acid Phosphatase Activity (Score/Plasma Cell)

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of Patients</th>
<th>Mean Acid Phosphatase Activity (AP Value ± Standard Deviation)</th>
<th>Mean Number of Acid Phosphatase Positive Cells (% ± Standard Deviation)</th>
<th>Patients With AP Score* of &gt;3 per Cell (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior to induction</td>
<td>8</td>
<td>3.06 ± 0.53</td>
<td>95.67 ± 9.78</td>
<td>55%</td>
</tr>
<tr>
<td>Remission</td>
<td>10</td>
<td>1.81 ± 1.02‡</td>
<td>75.55 ± 8.69‡</td>
<td>0%‡</td>
</tr>
<tr>
<td>Relapse</td>
<td>19</td>
<td>2.48 ± 0.77‡</td>
<td>93.38 ± 11.93</td>
<td>14.3%</td>
</tr>
</tbody>
</table>

*See Materials and Methods section.
†Significantly different to preinduction group, p < 0.01.
‡Significantly different to preinduction group, p < 0.05.
§Significantly different to preinduction group, p < 0.02.

### Table 2. Plasma Cell Acid Phosphatase Score/Plasma Cell and M-Component Kappa (ε)/Lambda (λ) Subtype

<table>
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<tr>
<th>Category</th>
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</table>

*All values significantly different, p < 0.05.
†Significantly different to ε remission values, p < 0.05.
such patients with low AP activity were in remission. Our preliminary results indicate that a high AP activity is found in untreated patients and those patients in relapse, whereas low activity is seen in remission (see Table 1). Serial studies in individual patients have shown transition from initial high acid phosphatase scores to low scores in remission and from low scores in remission to higher scores at the time of relapse. This supports the conclusion that the changes seen reflect disease status in individual patients rather than completely different patient populations. The finding of low AP scores in remission as opposed to pretreatment or at relapse was particularly true for kappa as compared to lambda MM, which retained a strong AP activity even in remission. The high AP activity in lambda patients, even in apparent remission, is particularly interesting in view of the shorter remissions and poorer survival reported for patients with lambda as opposed to kappa M-component subtype. Therefore, assessment of AP in MM may prove to be useful, not only in diagnosis, as suggested by Cassuto, but also to determine disease activity.

Further investigations will be necessary to assess the real importance of these findings, in particular, serial studies in individual patients will be required. However, it is already well known that in lymphoproliferative diseases, such as Waldenstrom’s macroglobulinemia, β-glucuronidase activity in tumor cells is low before any treatment and increases to normal levels in remission. Furthermore, it has also been documented that AP activity is increased in normal lymphocytes and macrophages after activation and in peripheral lymphocytes in MM. Therefore, changes of AP activity in myeloma cells during induction treatment, remission, and relapse might relate to cell activity. It would be interesting to correlate AP activity changes with other cytochemical markers of MM, such as high nonspecific esterase activity and low ATP-ase activity. In a few cases of myeloma, AP has been noted to be increased in serum or in bone marrow at the time of initial diagnosis, becoming normal in remission. These isolated observations may mean that AP in tumor cells and even in the serum may be useful as marker of disease activity in MM.

However, relationships between high AP and the functions of myeloma cells remain unclear. Acid phosphatase and β-glucuronidase are lysosomal enzymes, and normal bone resorption depends on the activity of such osteoclastic enzymes. Tumors with extensive bone involvement (osteolytic lesions), such as breast cancer, histiocytosis, Gorham disappearing bone disease, and giant cell tumors have a high AP activity with an extratumoral release of enzyme activity in a few cases. On the other hand, AP activity and β-glucuronidase activity are normal or low in chronic lymphocytic leukemia and Waldenstrom’s macroglobulinemia, where bone involvement is unusual. It was therefore logical to think that such high enzyme activity in myeloma cells might be involved in disease spread and bone lesions. However, in our present study, we found no correlation between AP activity and bone involvement or hypercalcemia.

In conclusion, our preliminary results confirm the potential importance of AP activity in MM, not only for diagnosis, but also for prognosis. Further investigations will be necessary to more fully appreciate the significance of acid phosphatase activity in MM.

ACKNOWLEDGMENT

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