Muramidase in Polycythemia Vera

BY RICHARD A. BINDER AND HARRIET S. GILBERT

The serum muramidase levels of 45 patients with polycythemia vera were found to be significantly elevated above the values in 20 normal subjects. The patients with polycythemia vera who were studied included those with active disease, those controlled with myelosuppressive agents and those in thespent phase. A high degree of correlation was found between muramidase levels and leukocyte count, granulocyte count, serum uric acid, serum vitamin B₁₂ content and unsaturated vitamin B₁₂ binding capacity. No correlation was found between muramidase levels and hemato crit, monocyte count and leukocyte alkaline phosphatase activity. It is felt that this study lends support to the origin of serum muramidase from granulocytes and that elevated levels of serum muramidase in the subjects studied are a reflection of the involvement of the granulocyte in the proliferative process responsible for polycythemia vera.

MURAMIDASE (LYSOZYME), A BACTERIOLYTIC HYDROLYTIC ENZYME, originally described by Fleming in 1922 recently has been found to be elevated in serum and/or urine in a number of diseases, including several disorders of the hematopoietic system such as megaloblastic anemia, monocytic and monomyelocytic leukemia, polycythemia vera, and acute myelogenous leukemia. In each of these disorders, the increased levels of enzyme have been attributed to an increase in the production and/or turnover of leukocytes, primarily monocytes and granulocytes.

The aim of this study was to confirm the presence of increased muramidase in polycythemia vera and to study the relationship of its level to other laboratory features of this disorder.

MATERIALS AND METHODS

The sera from 65 subjects were studied, 20 controls who were without hematologic or other disease, and 45 patients with polycythemia vera (without renal disease) who were being followed in the Polycythemia Vera Research Clinic of the Mount Sinai Hospital. Urine studies were discontinued after no Muramidase was detected. The diagnosis of polycythemia had been made on the basis of physical and laboratory findings, including some or all of the following: increased red cell mass, splenomegaly, leukocytosis, thrombocytosis,
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Fig. 1.—Comparison of serum muramidase values in normal subjects and patients in various stages of polycythemia vera (P.V.).

elevated leukocyte alkaline phosphatase activity (Kaplow method), elevated serum $B_{12}$ content and/or unsaturated $B_{12}$ binding capacity (UBBC).

The cases of polycythemia vera (P.V.) were staged on the basis of their erythroid activity as follows:

P.V. active: patients with increased erythroid activity, as evidenced by a rising hematocrit, who had received no recent treatment other than phlebotomy. This group was composed of newly diagnosed patients who had never been treated and patients in relapse who had received myelosuppressive therapy in the past.

P.V. controlled: patients in whom the hematocrit had stabilized at 52 per cent or below, without phlebotomy, in response to myelosuppressive therapy (agents used were chlorambucil, cyclophosphamide, busulfan and $^{32}$P).

Spent P.V.: patients with documented polycythemia vera whose hematocrit had stabilized at normal or low levels in the absence of bleeding or myelosuppressive treatment for a period of at least 3 years.

Muramidase levels were assayed for this study using the technique of agar diffusion. Assay plates were prepared from agarose in which *Micrococcus lysodeikticus* bacteria were suspended. Test samples and known dilutions of lyophilized human enzyme (kindly provided by Dr. Elliot Osserman) were introduced into wells of fixed volume. The enzyme activity of the samples was determined by the size of the zone of clearing surrounding the well, since the diameter of the clear zone (mm.) has been found to be directly proportional to the log of the concentration ($\mu$g/ml) of the enzyme present. Standard curves were made for each series of assays. The plates were read and photographed after 18–24 hours. Tests were performed on fresh sera and sera that had been stored at $-20^\circ$C. No diminution of enzyme activity was observed following storage for a period of 3 months.

Simultaneous determinations of white blood cell count and differential, hematocrit, leuko-
Table 1.—The Relation of Serum Muramidase Levels to Other Abnormalities in Myeloproliferative Disease

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. Cases</th>
<th>Linear Correlation Coefficient</th>
<th>p value of Linear Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit</td>
<td>45</td>
<td>-0.1397</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Leukocyte count</td>
<td>45</td>
<td>0.44</td>
<td>&lt;0.0025</td>
</tr>
<tr>
<td>Granulocyte count</td>
<td>45</td>
<td>0.41</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Monocyte count</td>
<td>45</td>
<td>-0.003</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Uric acid</td>
<td>29</td>
<td>0.50</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Serum B12</td>
<td>39</td>
<td>0.36</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Serum B12 binding protein</td>
<td>39</td>
<td>0.45</td>
<td>&lt;0.0025</td>
</tr>
<tr>
<td>Leukocyte alkaline phosphatase activity</td>
<td>36</td>
<td>0.26</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

cyte alkaline phosphatase activity, serum uric acid, serum vitamin B12 content and unsaturated vitamin B12 binding capacity (UBBC) were performed. Total granulocyte and monocyte counts were calculated from the white blood cell count and differential. The usual t statistic was used for testing the significance of differences between group means, as well as for testing the significance of the computed product moment correlation coefficients.10

RESULTS

The results of serum muramidase determinations in the groups studied are presented in Fig. 1. As compared with the mean value + 1 SD in 20 normal subjects (9.2 ± 4.5 µg./ml.), serum lysozyme levels were found to be significantly elevated in 20 patients with active polycythemia vera (13.3 ± 6.2 µg./ml., p < 0.01), in 19 patients with polycythemia vera being controlled with myelosuppressive therapy (12.7 ± 7.0 µg./ml., p < 0.05), and in six patients with the spent phase of polycythemia vera (15.8 ± 9.2 µg./ml., p < 0.01). In 14 of the 45 patients with polycythemia vera who were studied (31%), muramidase levels were greater than 13.7 µg./ml. (the normal mean + 1 SD).

The relation of serum muramidase levels to the other parameters studied is shown in Table 1. A high degree of correlation was found between muramidase level and the simultaneously measured white blood cell count, total granulocyte count, serum uric acid, serum B12 content and unsaturated B12 binding capacity. No correlation was found between serum muramidase level and hematocrit, total monocyte count or leukocyte alkaline phosphatase activity.

DISCUSSION

The specific cell type or types giving rise to muramidase has not been definitely established. There is good evidence that, in the presence of increased monocytic proliferation, marked elevation of muramidase can be found in both urine and serum.3 Others have demonstrated a strong correlation between serum muramidase levels and peripheral granulocyte count and/or granulocyte turnover.2,4-7 All studies demonstrate or postulate that muramidase is derived primarily from the degradation of mature and im
mature leukocytes. In this study patients with polycythemia vera (P.V.) were found to have serum muramidase levels significantly above normal. In all patients with P.V., a significant correlation was found between serum muramidase levels and the circulating granulocyte count. A similar finding has been described by Finch et al. This appears to support the postulate that, in this disease, granulocytes are the prime source of serum muramidase.

Cellular breakdown is said to be necessary for the release of muramidase into serum. Uric acid, the end product of nucleoprotein degradation, is commonly elevated in the myeloproliferative disorders as well as in leukemia, presumably due to increased cellular breakdown. The high degree of correlation shown to exist between serum muramidase levels and serum uric acid in this study lends further weight to the hypothesis that increased cellular breakdown is responsible for elevations of serum muramidase observed in polycythemia vera.

Since increased red cell production is a prominent feature of the panmyelosis of polycythemia vera, the relation of the hematocrit to muramidase levels was examined. The poor correlation between muramidase levels and the hematocrit in the patients studied, and the failure of phlebotomy to alter these levels in single individuals studied serially, make it unlikely that increased erythrocyte turnover is responsible for elevated serum muramidase. This is as expected since no muramidase has been demonstrated to be present in cells of the erythroid series.

Further evidence that muramidase is derived from leukocytes and, more specifically, granulocytes in P.V. is the high degree of correlation observed between muramidase and serum B12 and UBBC levels. Serum B12 and UBBC have been found to be elevated in polycythemia vera and it is believed that a significant portion of the vitamin B12 binding protein is derived from granulocytes.

In general, leukocyte alkaline phosphatase activity has been found to be elevated in all stages of polycythemia vera, but wide variations from high to low occur. Values for individual patients tend to remain within a fairly narrow range in spite of wide fluctuations in other parameters, i.e., uric acid, leukocyte count and hematocrit. Since the relationship of the leukocyte alkaline phosphatase to white cell levels or turnover is questionable, particularly in treated cases, one would not expect a correlation with muramidase levels in the population studied.

It is felt by some that muramidase is derived primarily from monocytes. Since our observations and those of others who have evaluated bone marrow and peripheral blood of patients with polycythemia vera show no increase in monocytes, it appears likely that the source of increased muramidase is the granulocyte and that the increased levels of muramidase observed are related to involvement of the granulocyte in the proliferative process. Although statistically significant elevations in muramidase levels were observed in polycythemia, this finding is felt to be nonspecific for myeloproliferative disease and, therefore, not of diagnostic significance.
REFERENCES


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