

## Serum Muramidase in Patients with Neutropenia\*

WESLEY M. VIETZKE,† PASQUALE E. PERILLIE,‡  
and STUART C. FINCH§

*Department of Internal Medicine, Yale University School of Medicine,  
New Haven, Connecticut 06510, and the Veterans' Administration Hospital,  
West Haven, Connecticut*

*Received for publication 29 November 1971*

The purpose of this study was to determine whether variations in serum muramidase (lysozyme) activity in neutropenic patients have clinical significance. The possibility of a valid relationship is based on the premise that most of the normal plasma enzyme activity is derived from the degradation of senescent neutrophils with subsequent release of enzyme from their granules(1-4). There now is abundant evidence that muramidase is found predominantly in the lysosomes of mature neutrophils and monocytes(5), and that the number of circulating mature neutrophils correlates well with serum muramidase activity(2-4). It also has been demonstrated that increased serum muramidase activity is associated with expanded total granulocyte or monocyte populations in patients with leukemia(1,3,6,7) and the probable increase in marrow granulocyte turnover in megaloblastic anemias(8). On the other hand, serum enzyme activity frequently is reduced in acute lymphocytic leukemia in relapse(7) and other conditions which appear to reduce the total body neutrophil population(1,3). These observations suggest that neutropenia due to diminished granulopoiesis may result in reduced serum muramidase activity whereas neutropenia due to accelerated granulocyte destruction may produce either a relative or absolute increase in serum enzyme activity. Preliminary observations in patients with aplastic anemia and various types of neutropenic disorders are consistent with this suggestion(3,9).

\* This research was supported by USPHS Training Grant HE05316-10 and USPHS Research Grant CA-11106.

† Formerly a Research Trainee in Hematology, Yale University School of Medicine, New Haven; currently an Assistant Professor of Medicine, University of Connecticut School of Medicine, McCook Hospital, Hartford, Ct. 06112.

‡ Associate Clinical Professor of Medicine, Yale University School of Medicine, New Haven, Ct.

§ Professor of Medicine, Yale University School of Medicine, New Haven, Ct.

## MATERIALS AND METHODS

Serum muramidase activity was measured in 55 neutropenic patients at either the Yale-New Haven Hospital or the West Haven Veteran's Administration Hospital. The peripheral blood neutrophil count was less than 2000/mm<sup>3</sup> in each patient at the time of study. A peripheral blood monocyte concentration of greater than 750/mm<sup>3</sup> was defined as monocytosis. All patients in the study had blood urea nitrogen levels below 20 mg/100 ml.

Serum muramidase activity for each patient was correlated with bone marrow granulocyte cellularity, peripheral blood neutrophil and monocyte counts, and clinical diagnosis. The neutropenia was due to a wide variety of clinical conditions, but for purposes of this study the degree of bone marrow granulocyte cellularity rather than disease diagnosis received primary consideration. Bone marrow granulocyte cellularity was determined from coverslip smears of marrow particles and bone marrow needle biopsy sections. The combination of overall marrow cellularity and the myeloid:erythroid ratio provided the basis for judging the extent of marrow granulocyte cellularity. The distribution of patients according to clinical diagnosis and marrow granulocyte cellularity is shown in Tables 1 and 2.

Serum muramidase activity was determined by a modification of Litwack's turbidimetric method using an egg white standard, measuring change in optical density of a suspension of *Micrococcus lysodeikticus* at 540 nm(10). Control values range from 7 to 14  $\mu\text{g/ml}$ (2,3).

## RESULTS

Serum muramidase activity was within or above the control range in all patients with normal or increased bone marrow granulocyte cellularity (Fig. 1, Table 1). Twelve of the 22 patients in this group (55%) had increased serum muramidase activity. The median serum value was 14  $\mu\text{g/ml}$  with a mean of 15.2  $\mu\text{g/ml} \pm 6.0$ . There were four patients in this group with peripheral blood

TABLE 1  
DISTRIBUTION OF PATIENTS WITH NEUTROPENIA ASSOCIATED WITH NORMAL OR INCREASED  
BONE MARROW GRANULOCYTE CELLULARITY ACCORDING TO DIAGNOSIS  
AND SERUM MURAMIDASE ACTIVITY

| Diagnosis                      | Serum muramidase ( $\mu\text{g/ml}$ ) |      |     | Total |
|--------------------------------|---------------------------------------|------|-----|-------|
|                                | <7                                    | 7-14 | >14 |       |
| Idiopathic neutropenia         | 0                                     | 2    | 5   | 7     |
| Hyperplastic refractory anemia | 0                                     | 1    | 2   | 3     |
| Lupus erythematosus            | 0                                     | 0    | 1   | 1     |
| Felty's syndrome               | 0                                     | 2    | 2   | 4     |
| 1° or 2° splenic hyperplasia   | 0                                     | 5    | 2   | 7     |
| Total                          | 0                                     | 10   | 12  | 22    |

monocytosis, each of whom had increased serum muramidase activity (Fig. 1). The median value for the patients with monocytosis was 16  $\mu\text{g/ml}$  and the mean value was 17.1  $\mu\text{g/ml} \pm 4.8$ . Increased serum muramidase was observed in eight of the remaining 18 patients (44%) with normal or increased marrow granulocyte activity but without monocytosis (Fig. 1). The median serum enzyme activity was 12.7  $\mu\text{g/ml}$  and the mean serum value was 14.8  $\mu\text{g/ml} \pm 5.1$ .

Serum muramidase activity for all patients with diminished marrow granulocyte cellularity was either within the control range or reduced (Fig. 1, Table 2). The median value was 4.0  $\mu\text{g/ml}$  with a mean of 5.5  $\mu\text{g/ml} \pm 3.3$  for this entire group. Serum enzyme activity was below the control range in 26 of 33 patients (79%). None of the patients with reduced marrow granulocytes had peripheral blood monocytosis.

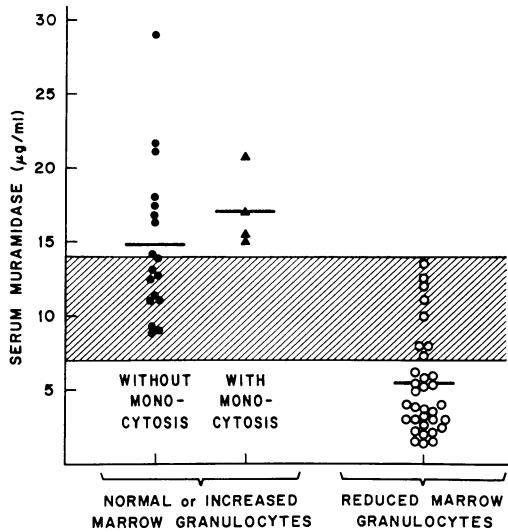


FIG. 1. This figure demonstrates that serum muramidase activity is normal or increased in all patients with monocytosis ( $\blacktriangle$ ) or without monocytosis ( $\bullet$ ) whenever marrow granulocyte cellularity is normal or increased. Neutropenia in the presence of reduced marrow granulocyte precursors ( $\circ$ ), however, is associated with normal or reduced serum enzyme activity. The control range for serum muramidase is shown by the crosshatched area and the median for each group by a horizontal bar.

TABLE 2  
DISTRIBUTION OF PATIENTS WITH NEUTROPENIA ASSOCIATED WITH DECREASED BONE MARROW GRANULOCYTE CELLULARITY ACCORDING TO DIAGNOSIS AND SERUM MURAMIDASE ACTIVITY

| Diagnosis                    | Serum muramidase ( $\mu\text{g/ml}$ ) |      |     | Total |
|------------------------------|---------------------------------------|------|-----|-------|
|                              | <7                                    | 7-14 | >14 |       |
| Aplastic anemia              | 10                                    | 4    | 0   | 14    |
| Drug-induced agranulocytosis | 5                                     | 2    | 0   | 7     |
| Radiation therapy            | 2                                     | 1    | 0   | 3     |
| Acute lymphocytic leukemia   | 8                                     | 1    | 0   | 9     |
| Total                        | 25                                    | 8    | 0   | 33    |

Comparison of the two groups indicates that reduced serum muramidase activity is more characteristic of those patients with reduced marrow myeloid cellularity (79%) than is increased serum muramidase activity for the group with normal or increased marrow granulocyte cellularity (55%). The difference between the mean serum muramidase value for those patients with diminished bone marrow myeloid activity and those with normal or increased myeloid activity (without monocytosis) is highly significant ( $P < 0.001$ ).

The overall relationship for either group between serum muramidase activity and peripheral blood neutrophil count was poor (Fig. 2). All but one patient with severe neutropenia, however, with peripheral blood neutrophil counts less than  $200/\text{mm}^3$  in association with reduced marrow granulocyte cellularity had reduced serum enzyme activity. For any given peripheral blood neutrophil count serum muramidase activity was increased in patients with monocytosis, but no greater than in some without monocytosis (Fig. 2). Thus, the relationship between serum muramidase and total blood monocytes or the combination of neutrophils and monocytes was poor for these extremely neutropenic subjects.

## DISCUSSION

These data demonstrate clear differences in leukocyte-muramidase relationships in patients with various types of neutropenia. First, those patients with normal or increased numbers of marrow granulocytes in whom there probably is increased neutrophil turnover have normal or increased serum muramidase activity. It seems likely that the relatively large amount of serum enzyme activity

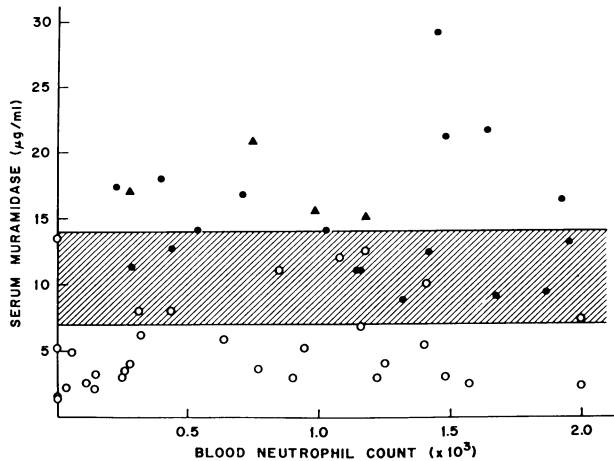


FIG. 2. A poor overall relationship is noted between blood neutrophils and serum muramidase. All patients with monocytosis ( $\blacktriangle$ ) had increased serum enzyme activity, but to levels which were no higher than those of many patients with increased marrow granulocytes without monocytosis ( $\bullet$ ). All but one patient with reduced marrow granulocytes and less than 300 circulating neutrophils had reduced serum muramidase activity. The crosshatched area shows the control range.

in the patients is due to increased neutrophil destruction. In the patients with monocytosis it is uncertain as to whether the increased serum muramidase activity is primarily of neutrophilic or monocytic origin, but the consistently higher levels of activity suggest a major monocyte contribution. The second group consists of patients with neutropenia associated with reduced marrow myeloid cellularity which is probably a reflection of decreased total granulopoiesis. Serum muramidase activity usually is reduced, but may be in the normal range. These observations suggest that, in the absence of monocytosis, increased serum muramidase activity in patients with neutropenia is related to active granulopoiesis in association with increased granulocyte destruction. Reduced serum muramidase activity is due to myeloid hypoproliferation resulting in diminished granulocyte turnover. Reduction of serum muramidase activity to levels as low as 1.4–1.6  $\mu\text{g/ml}$  occurred in patients with marrow myeloid hypoplasia or aplasia. This indicates that as much as 80% of serum muramidase activity is derived from de-graded granulocytes.

The "muramidase index" has proved of rather limited value in characterizing the type of neutropenia in this group of patients(7,8). If one excludes patients with severe neutropenia ( $< 200$  neutrophils/ $\text{mm}^3$ ), all of whom have reduced marrow granulocyte activity, the general trend is for higher indices in patients with normal or increased marrow granulocytes in comparison to those with reduced marrow granulocytes, but overlapping of values is too great to be of much clinical value.

The extent to which monocytes contribute muramidase to the body's total muramidase pool is difficult to ascertain. The relative number of monocytes in the peripheral blood of neutropenic patients frequently parallels the peripheral neutrophil count, especially when there is marrow myeloid hypocellularity. Within the decreased or normal range of blood monocyte concentrations, the monocyte-muramidase relationship is poor. Monocytosis, however, usually is associated with increased serum muramidase activity(1,2,6,7) so that the actual enzyme contribution from neutrophils in the presence of increased numbers of monocytes may be relatively small. Furthermore, there always is the problem as to whether or not the peripheral blood monocyte count reflects the size of the total body monocyte pool. Some patients with monocytic leukemia have low peripheral blood monocyte counts in the presence of marrow monocytosis and increased serum muramidase activity(6). A similar phenomenon cannot be excluded as the cause for normal or increased serum muramidase activity in some of our most neutropenic patients.

It is of interest that all neutropenic patients with peripheral blood monocytosis had normal or increased bone marrow-myeloid activity. Not only is this of possible diagnostic importance in terms of granulocyte mechanisms in patients with neutropenia, but there may be important implications concerning a common etiology for these cells. In accord with this relationship is the fact that monocytopenia occurred in the patients with reduced marrow granulocytic activity and the most severe reductions in peripheral blood neutrophils.

The close correlation between serum muramidase and bone marrow granulocyte activity in neutropenic patients may provide another useful parameter for the laboratory study of the mechanism of neutropenia in some patients. Decreased serum muramidase activity invariably reflects decreased neutrophil production. Increased serum muramidase in the absence of azotemia reflects either increased neutrophil turnover or monocytosis. Serum muramidase activity in the control range adds little information of value to the study of these. The eventual clinical usefulness of these relationships in the diagnosis and management of neutropenic patients, however, is uncertain at this time due to overlapping of values in some instances and minimal change in others.

### SUMMARY

Serum muramidase activity was related to bone marrow and peripheral blood changes in 55 patients with various types of neutropenia. Normal or reduced serum enzyme activity was observed in all patients with diminished bone marrow granulocyte cellularity. Patients with neutropenia associated with abundant bone marrow granulocytes generally had normal or increased serum muramidase activity.

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