Serum Muramidase and Granulocyte Turnover* (32692)

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Both quantitative cell assay studies and direct histobacterial techniques have demonstrated the presence of muramidase (lysozyme) in cells of the granulocytic and monocytic series (1–4). These same techniques have failed to demonstrate such enzyme activity in lymphocytes, eosinophils, and basophils. The relationship between serum muramidase and circulating leukocytes in a healthy adult population has led to the suggestion that serum muramidase is derived primarily from the steady destruction of senescent granulocytes (5). Observations in our laboratory on serum muramidase activity in patients with a variety of hematologic disorders has suggested that measurement of this enzyme in the serum might provide a valuable means of assessment of the rate of granulocyte turnover. In order to test this hypothesis, the effect of a gradual reduction in granulocyte mass on serum muramidase was determined in nitrogen mustard treated rabbits. In addition, the acute change in serum muramidase in rabbits following rapid and massive destruction of circulating granulocytes due to heterologous granulocyte antibody was studied.

Materials and Methods. In all studies, serum muramidase levels were determined by a modification of the method of Smollelis and Hartsell (6). This assay technique depends upon the measurement of change in optical density produced by an aliquot of test serum on a standardized turbid suspension of the organism *Micrococcus lysodeikticus*. Muramidase activity was expressed per ml of serum in crystalline egg white muramidase microgram equivalents.

In the first series of experiments a single 1.6 mg/kg dose of nitrogen mustard[1] was injected into a marginal ear vein of six female New Zealand rabbits. During the next week venous blood was obtained at frequent intervals for assay of muramidase activity along with total and differential leukocyte counts.

The second portion of the study involved evaluation of serum muramidase in rabbits injected with guinea pig antirabbit granulocyte serum. The antigenic granulocytes were obtained from rabbit peritoneal exudates. The inflammation producing solution consisted of an autoclaved mixture of 1 liter of normal saline, 1 gm of shell fish glycogen, 100,000 units of Penicillin G and 0.5 gm of streptomycin. A total of 450 ml of the mixture was infused over a 5-minute period into the peritoneal cavities of each of several 15-pound New Zealand rabbits. Twelve hours later the distended peritoneal cavities were aspirated and mixed with sodium EDTA under strict aseptic conditions. The granulocyte-rich exudates then were combined and centrifuged in a cold centrifuge at 1,500 rpm. The supernatant was discarded and the cellular sediment was washed once with 50 ml of normal saline. The exudates contained on the average 2.4×10^9 cells of which 92% were mature polymorphonuclear leukocytes. Saline suspensions of these cells were injected into all four foot pads of several guinea pigs, and following a good inflammatory response, they were given intradermal injections of the same cell suspensions at 2 to 3-day intervals until maximum arthus phenomena were noted. Sterile technique then was employed in the exsanguination of the guinea pigs and eventual pooling of the sera. The pooled serum had a slide agglutination titer of 1:640 against freshly obtained rabbit granulocytes. Two ml of the sterile, pooled serum was injected into a marginal ear vein of each of four New Zealand rabbits. Prior to injection, blood was removed by cardiac puncture for baseline levels of muramidase, total and differential leukocyte counts. These determinations were

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[1] Nitrogen mustard is manufactured as Mustargen Hydrochloride by Merck, Sharp and Dohme.

repeated at 30 and 60 min postinjection and at hourly intervals during the next 3 hours. Two control rabbits were studied in a similar fashion following intravenous injection of 2 ml of normal guinea pig serum. Another control study was designed to determine whether or not guinea pig antileukocyte serum altered the serum muramidase of a leukopenic animal. Two additional rabbits were injected with 2.5 mg/kg of nitrogen mustard and 55 hours later, when severe neutropenia had developed, each was given an intravenous injection of 2 ml of guinea pig antirabbit granulocyte serum. During the next 4 hours cardiac blood was obtained at frequent intervals for evaluation of serum muramidase as well as total and differential leukocyte counts.

Results. The effect of a gradual blood leukocyte reduction on levels of serum muramidase is shown in Fig. 1. Following injection of

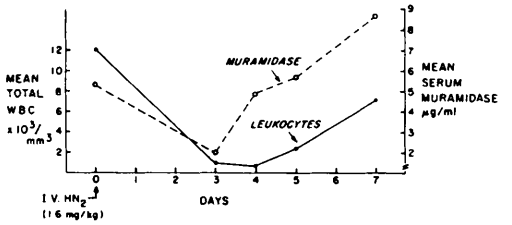

FIG. 1. Mean serum muramidase levels and mean total leukocyte counts of 6 rabbits following a single intravenous injection of nitrogen mustard are shown.

six healthy rabbits with 1.6 mg/kg of nitrogen mustard, the low point of total leukocyte depression occurred on the third and fourth postinjection days. At this time all of the rabbits showed absolute granulocyte counts below 700 cells/mm³. Serum muramidase fell concomitantly with the fall in total leukocyte count. Leukopenia persisted for several days and during the recovery phase serum enzyme activity increased 24–48 hours before there was a significant number of granulocytes in circulation. At the time serum muramidase had reached baseline levels, total leukocyte counts still were depressed.

The effects of massive acute destruction of circulating granulocytes on serum muramidase are shown in Fig. 2. Following rapid intravenous injection of guinea pig antirabbit granulocyte sera, granulocytopenia occurred

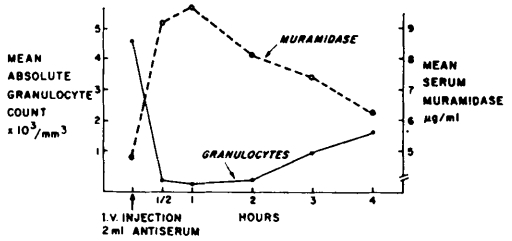

FIG. 2. The changes in mean serum muramidase and mean absolute granulocyte count are shown for 4 rabbits following intravenous injection of guinea pig antirabbit granulocyte serum.

within 0.5 hour in all rabbits. This profound granulocytopenia persisted for at least 4 hours. Concomitant with the onset of granulocytopenia, there was a prompt rise in serum muramidase, with peak levels at 1 hour following injection. Maximum serum enzyme cleared from the plasma in 4-5 hours. Neither granulocytopenia nor appreciable alteration of serum enzyme activity occurred following the administration of normal guinea pig serum to normal rabbits (Fig. 3).

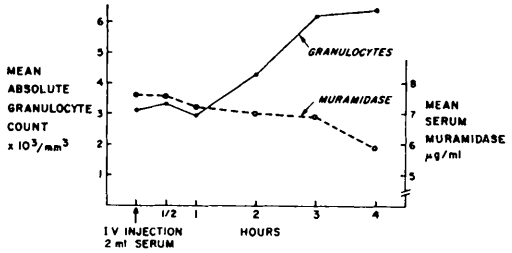

FIG. 3. Mean serum muramidase and mean absolute granulocyte count are shown for 2 rabbits following intravenous injection of normal guinea pig serum.

The nitrogen mustard induced granulocytopenic rabbits showed no significant increase in serum muramidase activity during the 4-hour period of observation following administration of antirabbit granulocyte serum (Fig. 4). At the time of antiserum administration, both animals had absolute granulocyte counts below 600 cells/mm³. The antiserum produced a slight further depression of granulocytes to levels below 100 cells/mm³.

Discussion. The data indicate that serum muramidase levels parallel the turnover of granulocytes in the peripheral blood. The gradual induction of granulocytopenia results

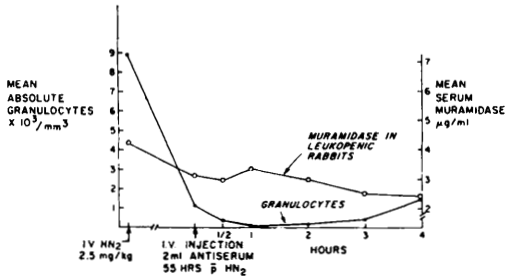

FIG. 4. The change in mean serum muramidase following injection of 2 rabbits rendered leukopenic with nitrogen mustard following injection with guinea pig antirabbit granulocyte serum.

in diminished serum enzyme activity. This is believed due to a reduction in the total granulocyte mass with consequent reduction in the number of senescent granulocytes which release enzyme during cell degradation. In contrast, acute reductions in granulocyte mass, as accomplished by administration of antigranulocyte serum, cause an abrupt increase in levels of serum muramidase. This presumably is due to the rapid destruction of a large mass of cells which released increased amounts of enzyme into the circulation. The magnitude of the serum muramidase change in the granulocytopenic rabbit is consistent with the suggestion that 70% or more of the serum muramidase is derived from the turnover of granulocytes under normal circumstances (2).

It is known that such viscera as kidney, liver, spleen, and lung contain appreciable amounts of muramidase. Since unabsorbed antiserum was used in these experiments, there was a possibility that the antiserum lacked specificity against granulocytic cells. Under these circumstances, a rise in muramidase would occur if viscera containing the enzyme were damaged and, in turn, released enzyme into the circulation. The failure of leukopenic animals to increase their serum enzyme levels following injection of antiserum, we believe satisfactorily excludes the possibility that visceral damage was responsible.

The increase in serum muramidase activity that precedes evidence of leukocyte recovery in the nitrogen mustard treated rabbit raised the possibility that the initial phase of bone marrow regeneration is characterized by a stage of ineffectual granulopoiesis. This is consistent with the observation that immature granulocytes may be present in the peripheral blood of patients with drug induced agranulocytosis during the first few days of marrow recovery. These observations lead to the suggestion that in acutely granulocytopenic patients the detection of either a rise in the serum muramidase level or the appearance of young granulocytes in the peripheral blood may be an early indication of marrow recovery.

The results of the acute agranulocytosis experiments indicate that the granulocytopenia following the injection of granulocyte antiserum probably is the result of intravascular leukocyte destruction rather than transient margination. The characteristics of the fall in the elevated serum enzyme level following acute granulocyte destruction suggests a muramidase $T_{½}$ in the range of 2–3 hours under these conditions. This clearance rate is somewhat slower than that reported by Perri *et al.* using egg white muramidase in rats (7). These studies provided only a crude assessment of muramidase turnover. More precise estimates probably will be derived only from clearance studies of isotope tagged homologous enzyme.

Summary. Studies in rabbits indicate that serum muramidase activity probably is derived mostly from the degradation of granulocytes. The data also suggest that serum muramidase levels may be a useful index of granulocyte turnover.

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