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A STUDY ON THE CLINICAL SIGNIFICANCE OF SERUM AND URINARY MURAMIDASE ACTIVITY IN LEUKEMICS

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ABSTRACT

A study was made on serum and urinary muramidase in 56 patients with leukemia and other hematologic disorders. Serum and urinary muramidase levels prior to antileukemic therapy were elevated in monocytic leukemia, while not so increased in paramyeloblastic leukemia. In all patients with acute lymphocytic leukemia pretreatment serum enzyme activities were decreased below normal, whereas those in patients with acute myelocytic leukemia were normal or slightly increased. In other hematologic diseases the mean values of serum muramidase levels before treatment were elevated slightly or moderately. Extreme urinary muramidase excretion was found in untreated patients with monocytic leukemia in contrast to none or little amount in the other groups. The serum and urinary muramidase levels changed in close correlation with peripheral leukocyte counts, bone marrow contents and clinical status in patients with monocytic leukemia. When antileukemic therapy was effective, serum and urinary muramidase levels fell toward the normal as leukemic cell counts decreased. During complete remission serum enzyme levels remained within normal range and muramidasuria was never found. Following relapse muramidase levels began to rise. Serum and urinary enzyme activities increased in a patient with monocytic leukemia in whom only a few blastic cells were found in peripheral blood and bone marrow.

On the basis of the present results, diagnostic value of serum and urinary muramidase activity and the relationship between the clinical status and the enzyme activity in leukemia, especially in monocytic leukemia, were discussed.

INTRODUCTION

It is very important to make a precise classification of leukemia, because of not only academic but also clinical interests[1]. The classification has so far been made chiefly based on the morphologic features of leukemic cells. Even with morphological techniques, however, it has been often difficult in some cases of leukemia to achieve clear-cut differentiation[2]. Especially, the criteria for monocytic leukemia have been in controversy[3)-8)] because of different opinions about the origin of monocytes[3)-7)9)].

Osserman and Lawlor[10] recently reported that serum and urinary muramidase

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levels in patients with monocytic leukemia were extremely elevated. This enzyme may be helpful for diagnosis of the leukemia as a biochemical parameter. As yet only a few investigations[11)12)] have been carried out on the enzyme alterations in correlation with the clinical status of patients with leukemia.

The present study was attempted to evaluate a diagnostic value of serum and urinary muramidase activity in untreated patients with leukemia, especially monocytic leukemia, and to estimate the significance of serial determination throughout the course of monocytic leukemia as a therapeutic and prognostic indicator.

MATERIALS AND METHODS

Clinical Materials. Serum and urinary muramidase was measured before treatment in 56 patients with acute and chronic leukemias and other hematlogic diseases, including 9 patients with acute myelocytic leukemia, 7 patients with chronic myelocytic leukemia, 7 patients with paramyeloblastic leukemia, 6 patients with acute lymphocytic leukemia, one patient with chronic lymphocytic leukemia, 14 patients with monocytic leukemia (one of them was a so-called "Schilling type"), 8 patients with multiple myeloma and 4 patients with malignant lymphoma. In addition to these patients, 54 patients with sarcoidosis were examined.

In ten patients with monocytic leukemia, serum and urinary muramidase levels were serially determined throughout their courses. At the same time peripheral leukocytes and bone marrow were examined. Volunteers from the laboratory and hospital staff and other apparently healthy subjects provided blood and urine for the normal control group.

Venous blood was withdrawn through a large gauge needle and allowed to clot at room temperature. Immediately after clotting, it was centrifuged at 3,000 r.p.m., for thirty minutes, and then the serum was separated with care to avoid hemolysis. Aliquots of twenty-four hour urine collection were used for urinary muramidase determinations. Samples which were not immediately used for the enzyme assay were stored at $-20°C$ for a week at the longest.

Diagnosis and Classification. Diagnosis was made based on physical examinations, peripheral hemograms and bone marrow aspirations. Leukemias were typed according to generally accepted criteria[2)13)14)], on the basis of circulating blood and bone marrow cell morphology, using the Giemsa stain, peroxidase and supravital staining reactions and phase contrast microscopy. In some cases phagocytic test was performed. The essential points of the criteria for monocytic leukemia were that more than 20 per cent of peripheral leukocytes were monocytes capable of phagocytosis and that transitional forms between monoblasts and mature monocytes were observed in bone marrow. Leukemic cells of paramyeloblastic leukemia had no mobility and no intermediate form

to monocyte, even if they had an overlapped or indented nucleus. Moreover, increased numbers of mature monocytes were never found in peripheral blood and bone marrow in these cases.

Determination of Muramidase Activity. Serum and urinary muramidase levels were measured by the lysoplate method employing heat-killed *Micrococcus lysodeikticus* organisms incorporated in agar as originally developed by Osserman and Lawlor[10] and corroborated by Zucker *et al.*[17]

Human muramidase was isolated and purified from the urine of patients with monocytic leukemia according to the procedure of Alderton, Ward and Fevold[15,16] with minor modification, using bentonite adsorption and elution with 5 per cent aqueous pyridine adjusted to pH 5.0 with sulfuric acid. Then lyophilized muramidase was crystallized at pH 10.5. This preparation had eight times greater activity than hen's egg-white enzyme (Sigma), and its dilutions (5, 10, 50, 100, 500 and 1,000 μg per ml) were used as standard.

RESULTS

Serum Muramidase Levels before Treatment. Individual serum muramidase levels before treatment are presented in Fig. 1. These values were the maximum when measured more than twice in each case. Control value for serum muramidase in 35 normal subjects ranged from 4.0 to 9.0 μg per ml with a mean of 7.0±1.3 (S.D.) μg per ml. From this result the normal range in this report was established to be 4.0 to 9.0 μg per ml. Mean values and ranges for each untreated group were as follows: (Table 1) acute myelocytic leukemia 8.9±4.0 μg per ml (range 2.7 to 14.5 μg per ml), chronic myelocytic leukemia 17.5±5.7 (4.5 to 26.0), paramyeloblastic leukemia 11.6±8.5 (4.5 to 29.0), acute lymphocytic leukemia 2.9±1.2 (1.2 to 4.6), chronic lymphocytic leukemia 4.0,

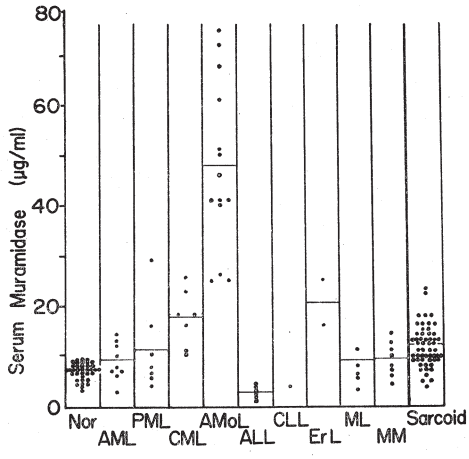

FIG. 1. Scattergram of serum muramidase levels before treatment in patients with leukemia and some other hematologic diseases. Points represent serum muramidase values for individual patients. Open circle represents serum muramidase value of patient with Schilling type monocytic leukemia. Horizontal bars represent mean values for each group. Nor=normal control, AML=acute myelocytic leukemia, PML=paramyelocytic leukemia, CML=chronic myelocytic leukemia, MoL=monocytic leukemia, ALL=acute lymphocytic leukemia, CLL=chronic lymphocytic leukemia, ErL=erythroleukemia, ML=malignant lymphoma, MM=multiple myeloma, Sarco=sarcoidosis.

TABLE 1. Serum Muramidase Levels in Various Hematologic Diseases (before treatment)

Disease	No. of cases	Serum muramidase levels (μg/ml)	
		Mean±(S.D.)	Range
Normal	35	7.0± 1.3	4.0– 9.0
Acute myelocytic leukemia	9	8.9± 4.0	2.7–14.0
Paramyeloblastic leukemia	7	11.6± 8.5	4.5–29.0
Chronic myelocytic leukemia	7	17.5± 5.7	10.5–26.0
Monocytic leukemia	14	47.8±17.3	25.0–75.0
Acute lymphocytic leukemia	6	2.9± 1.2	1.2– 4.6
Chronic lymphocytic leukemia	1	4.0	
Multiple myeloma	8	9.1± 3.4	4.3–14.5
Malignant lymphoma	4	9.0± 2.4	6.0–11.0
Sarcoidosis	54	12.0± 4.3	4.0–23.0

monocytic leukemia 47.8±17.3 (25.0 to 75.0), multiple myeloma 9.0±2.4 (6.0 to 11.0), malignant lymphoma 9.1±3.4 (4.3 to 14.5) and sarcoidosis 12.0±4.3 (4.0 to 23.0). One patient with monocytic leukemia who was classified as so-called "Schilling type" by morphological features of leukemic cells had a level of 47.0 μg per ml. The levels in monocytic leukemia group were significantly greater than those in the control and the other groups (t-test $P<0.001$).

Urinary Muramidase Levels before Treatment (Table 2). Urinary muramidase excretion occurred only in some patients with paramyeloblastic leukemia, chronic myelocytic leukemia, monocytic leukemia, multiple myeloma and sarcoidosis. No activity in the urine was found in any control subjects or in

TABLE 2. Urinary Muramidase Levels in Various Hematologic Diseases (before Treatment)

Disease	No. of cases	Urine muramidase levels (mg/day)		No. patients with muramidasuria
		Mean	Range	
Normal	35	0		0
Acute myelocytic leukemia	9	0		0
Paramyeloblastic leukemia	7	0.2	(0–1.2)	2
Chronic myelocytic leukemia	7	0.6	(0–1.2)	4
Monocytic leukemia	14	802	(160–3,910)	14
Acute lymphocytic leukemia	6	0		0
Chronic lymphocytic leukemia	1	0		0
Multiple myeloma	8	0.4	(0–1.6)	2
Malignant lymphoma	4	0		0
Sarcoidosis	54		(0–6.8)	7

any patients with lymphocytic leukemia, acute myelocytic leukemia and malignant lymphoma. All patients with monocytic leukemia had markedly elevated urinary muramidase levels before treatment with a mean of 802 mg per day (range 160 to 3,910 mg per day). The patient with so-called "Schilling type" monocytic leukemia had a level of 2,370 mg per day. In two patients with paramyeloblastic leukemia urinary excretions were 1.2 mg and 0.2 mg a day. In four patients with chronic myelocytic leukemia enzyme levels were 1.9, 1.6, 0.5 and 0.3 mg a day respectively. Two patients with multiple myeloma had 1.6 and 1.2 mg muramidase in each urine per day. Only in seven patients with sarcoidosis muramidasuria was detected within the range of 0.4 to 6.8 mg per day. Before treatment all patients with monocytic leukemia had high levels of urinary muramidase at all times, but in other groups with maximum values as shown above, serial positive findings were rare in repeated examinations. No patient had urinary muramidase activity when the serum level fell below 20 μg per ml, but not all serum levels above this value were associated with muramidasuria.

All patients had no renal disorder nor serum electrolytes abnormality.

Serial Determinations of Serum and Urinary Muramidase Levels in Monocytic Leukemia. Complete remission was achieved in five patients in whom serial determinations were made. Three of them had a relapse during this study. Variations of serum and urinary muramidase levels and circulating and bone marrow leukocyte counts of a relapsed patient are shown in Fig. 2 (Case 1). The initial serum and urinary muramidase levels were 30.0 μg per ml and 240 mg per day respectively. These levels were the highest in his course. Induction

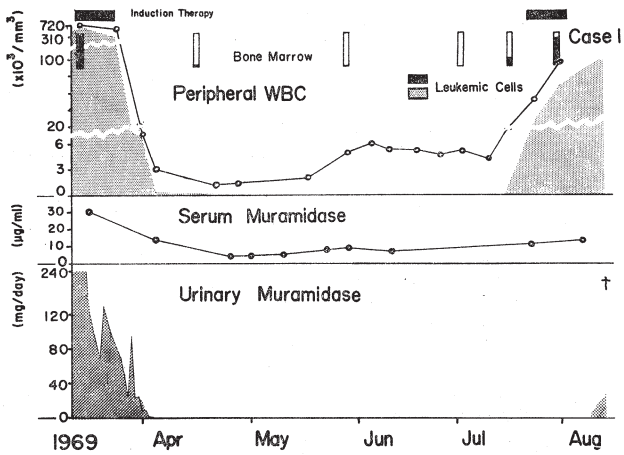

FIG. 2. (Case 1). Changes in serum and urinary muramidase levels of a patient with remitting and relapsing monocytic leukemia in relation to peripheral leukocyte counts and blood and marrow blast cells plus monocytes.

therapy caused peripheral leukocyte counts to fall progressively. According to this change serum muramidase levels reduced day by day and became normal in value a few days before remission was attained. Urinary excretion decreased more rapidly and could not be detected four weeks prior to complete remission induction. During complete remission serum muramidase levels remained within normal range and muramidasuria could not be found. It was one week after the initiation of relapse that serum muramidase activities began to increase. This delayed elevation was observed similarly in other two cases. Urinary muramidase excretion appeared again much later than the elevation in serum. Since then serum and urinary muramidase activities increased gradually but did not reach the initial levels.

Two patients, in whom complete remission was achieved, have not had relapse during this study. The course of one of these patients is presented in Fig. 3 (Case 2). His initial leukocyte count was 72,000 cells per cu. mm. Ninety-five per cent of the peripheral leukocytes and 98 per cent of total nucleated marrow cells consisted of monocytic series. Initial serum and urinary muramidase levels were 37.5 μg per ml and 150 mg per day respectively. During the next one week leukocyte counts increased progressively and reached 350,000 cells per cu. mm. Approximately 97 per cent of leukocytes were immature cells. Parallel with the increase in peripheral leukocyte counts, serum and urinary muramidase levels were markedly elevated to 50.0 μg per ml and 3,900 mg per day respectively. The changes after antileukemic therapy were similar to those of the first case. Good correlation between muramidase levels and leukocyte counts was observed throughout the clinical course of the five patients described above (Case 1 and 2).

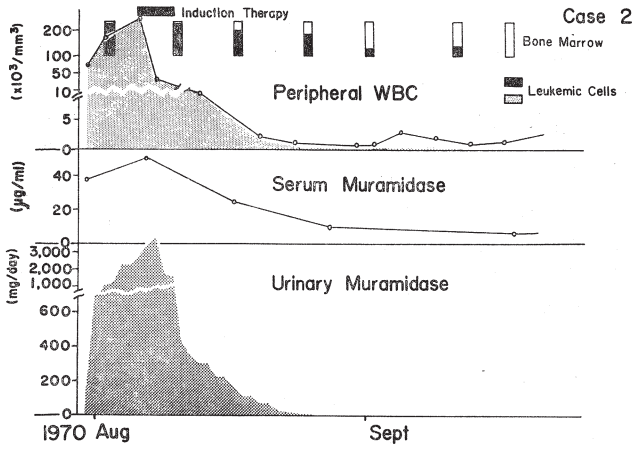

FIG. 3. (Case 2). Changes in serum and urinary muramidase levels of a patient with remitting monocytic leukemia in relation to peripheral leukocyte counts and blood and marrow blast cells plus monocytes.

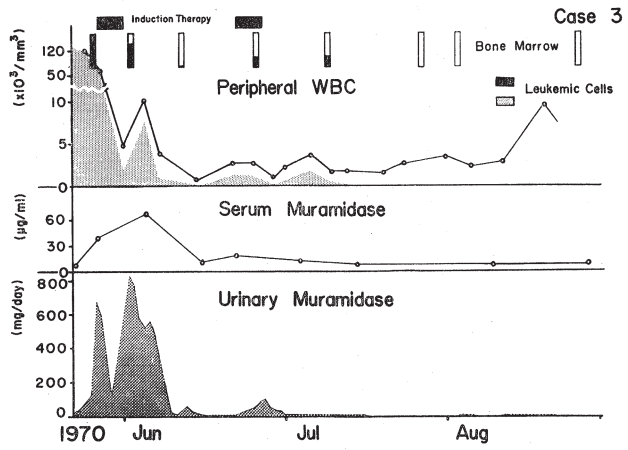

FIG. 4. (Case 3). Changes in serum and urinary muramidase levels of a patient with remitting monocytic leukemia in relation to peripheral leukocyte counts and blood and marrow blast cells plus monocytes.

The course of another patient who has been in complete remission is presented in Fig. 4 (Case 3). In the initial examinations serum and urinary muramidase levels were relatively of low degree, 18.0 μg per ml and 10.5 mg per day respectively, while peripheral leukocyte count was 120,000 cells per cu. mm. Immature monocytes and mature monocytes dominated in peripheral blood and bone marrow. Both serum and urinary activities, however, increased rapidly and reached the maximum within a week (40.0 μg per ml, 680 mg per day). Antileukemic chemotherapy resulted in a decrease of leukocyte counts and bone marrow blast cells with correlated reduction of serum muramidase levels. Urinary enzyme excretion directly reflected the decrease in number of circulating leukocytes. Thereafter, recurrence occurred twice before remission was achieved. During this period it was apparent that muramidase levels correlated very closely with the clinical status and leukocyte counts.

In spite of chemotherapy, complete remission did not occur in five patients. One of them, presented in Fig. 5 (Case 4), had high muramidase activities wich were stable both in serum and urine throughout his course. Peripheral and marrow leukemic cell counts did not decrease throughout his course.

In the other two patients antileukemic therapy produced a rapid drop in the white blood cell counts and the leukemic cells in bone marrow. Along with the hematological changes, the elevated muramidase levels returned to normal in serum and no excretion was found in urine. However, in some cases as exampled in Fig. 6 (Case 5), muramidase levels remained within normal range for some time, even though leukemic cells did not completely disappear from both peripheral blood and bone marrow. Serum muramidase activities

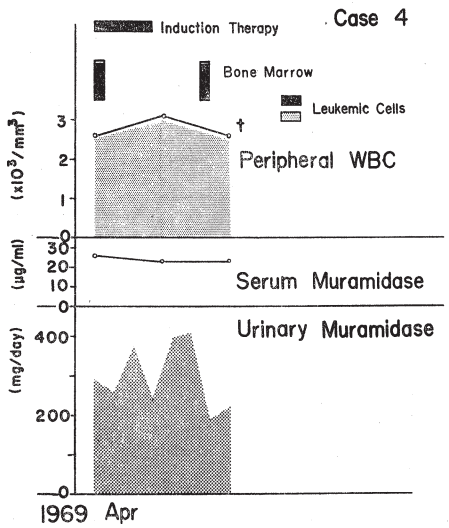

FIG. 5. (Case 4). Changes in serum and urinary muramidase levels of a patient with nonremitting monocytic leukemia in relation to peripheral leukocyte counts and blood and marrow blast cells plus monocytes.

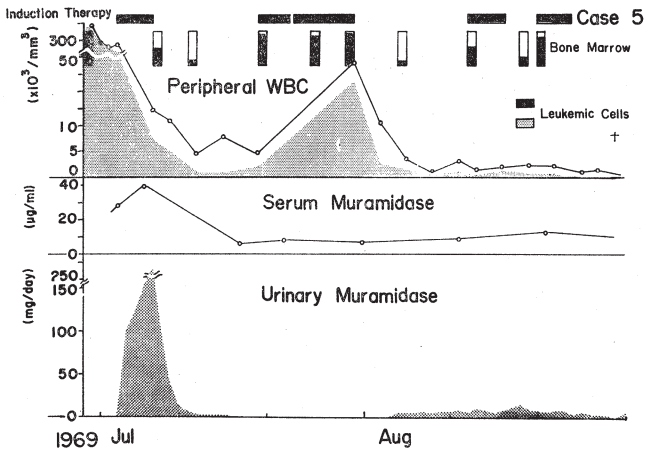

FIG. 6. (Case 5). Changes in serum and urinary muramidase levels of a patient with nonremitting monocytic leukemia in relation to peripheral leukocyte counts and blood and marrow blast cells plus monocytes.

began to increase gradually but several days after an apparent recrudescence occurred.

In the early stage, diagnosis was difficult morphologically in the two remaining patients without remission, though the initial serum and urinary muramidase levels were elevated in each case. The course of one of these

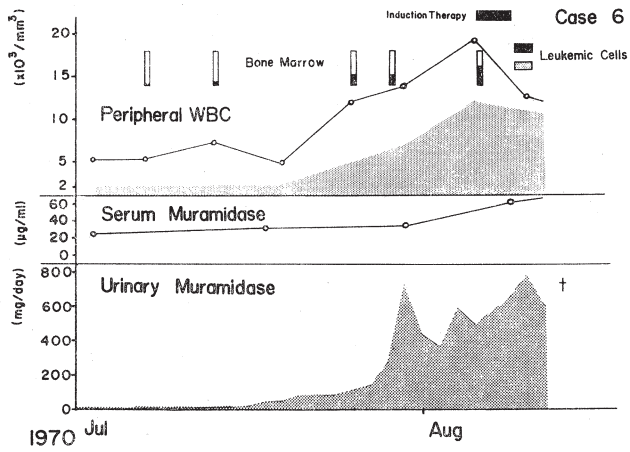

FIG. 7. (Case 6). Changes in serum and urinary muramidase levels of a patient with monocytic leukemia, lung tuberculosis and operated stomach cancer, in relation to peripheral leukocyte counts and blood and marrow blast cells plus monocytes. Monocytosis was remarkable but blast cells were only few in peripheral blood and bone marrow before the late stage.

patients is presented in Fig. 7 (Case 6). The hematological findings remained fairly constant in the early stage. Peripheral leukocyte count was 5,300 cells per cu. mm. of which 16 per cent were monocytes and 24 per cent were promonocytes. A few or no blastic cells were found in the peripheral blood. In the aspirated bone marrow specimen, the total nucleated cell count was 207×10^3 cells per cu.mm., of which 2.2 per cent were monocytes, 2.2 per cent promonocytes and 6.6 per cent blastic cells. Promonocytes in this case resembled monocytes in morphologic features having phagocytic activity. Cellularity of other series in bone marrow was almost normal. He had an old focus of pulmonary tuberculosis and had undergone gastrectomy for stomach cancer five years before. From these facts it was difficult to diagnose whether this monocytosis was a sign of a reactive monocytosis, leukemoid reaction or an early stage of monocytic leukemia. At this time serum and urinary muramidase activities were measured with results of 28.0 μg per ml and 14.4 mg per day respectively, which indicated a possibility of monocytic leukemia. During the early ten days no marked change occurred in the contents and counts of peripheral leukocytes and bone marrow cells, while serum and urinary muramidase activities increased slowly but steadily. Thereafter, circulating white blood cell counts, especially the absolute number of monocytes and promonocytes, increased gradually and in bone marrow monocyte series became approximately 30 per cent of total nucleated cells. On the sixteenth day of his course, marked elevation of urinary enzymy level occurred suddenly and reached 730 mg per day. At this time serum muramidase level was 38.0 μg

per ml. Peripheral leukocyte count was 13,800 cells per cu.mm. of which 3 per cent were blastic cells and 49 per cent the sum of monocytes and promonocytes. Serum muramidase activity continued to increase and finally reached 61 μg per ml. In urine the enzyme level remained high with some fluctuations. In the last bone marrow examination 24 per cent of nuclear cells consisted of blastic cells, although only 4 per cent of peripheral leukocytes were blastic. At this time the diagnosis of monocytic leukemia was confirmed by morphological findings.

Comparisions of the muramidase levels determined at the initial, the worst stage before treatment, remission and relapse in each patient with monocytic leukemia are presented in Fig. 8. Generally, the initial serum muramidase levels were lower than those at the worst stage but relatively close to each other. In remission serum levels of all patients returned to normal range. When relapse occurred serum enzyme activities increased again but did not reach the initial levels in all patients except one case whose serum level did not increase but remained normal. In contrast to serum enzyme activities, the initial urinary activities were low, ranging from 10.6 to 33.6 mg per day, in all but one patient whose urinary muramidase level was 285 mg per day. These low initial activities began to increase rapidly and to reach extreme high levels within several days. None of the patient excreted detectable amount of enzyme in urine during remission. In relapse enzyme activities increased again but the degree was low in all cases.

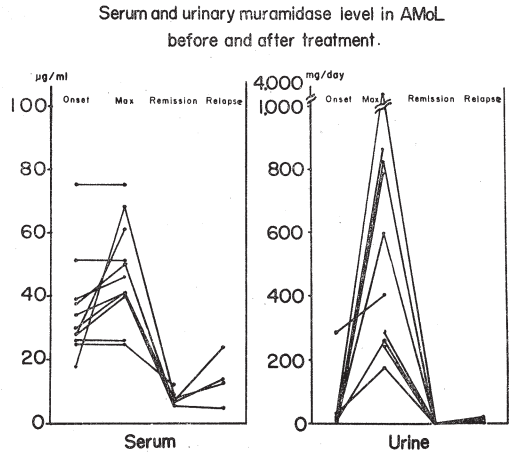

FIG. 8. Comparison of serum and urinary muramidase lovels determined at the initial and worst stages before treatment and remission and relapse in each patient with monocytic leukemia.

DISCUSSION

The results of this study indicate that pretreatment serum muramidase activities were markedly increased in patients with monocytic leukemia including so-called "Schilling type". These levels were significantly higher than those of any other groups. These results are in agreement with the reports of Jolles[18], Osserman[10] and Perillie[11].

On the other hand, in paramyeloblastic leukemia which generally has many diagnostic problems particularly in differentiation from monocytic leukemia by morphological observations[2], the pretreatment serum muramidase was lower than 20.0 μg per ml, except a patient who had a concentration of 29.0 μg per ml. Mean value in paramyeloblastic leukemia was 11.6 μg per ml in contrast to 47.0 μg per ml in monocytic leukemia. These findings suggest that determination of serum muramidase is helpful for differentiation between monocytic leukemia and paramyeloblastic leukemia.

Frequently, the differentiation between acute myelocytic leukemia and acute lymphocytic leukemia, entirely based on the morphologic features of leukemic cells, is quite difficult[2]. The present results, that serum muramidase activities decreased below normal in acute lymphocytic leukemia, while the levels remained within normal range or increased slightly in acute myelocytic leukemia, indicate the possibility that pretreatment serum muramidase activity is helpful for differentiation of the two types.

In earlier reports increased serum muramidase levels were observed in patients with various disorders other than leukemias characterized by proliferation or increased turnover of granulocytes and monocytes, such as tuberculosis[10], sarcoidosis[10], Hodgkin's disease[18], infectious diseases with neutrophilia[19], or megaloblastic anemia[20]. The degrees of increase of the serum enzyme levels, however, have been reported to be slight or moderate in these patients. Serum muramidase activity, however, remained nearly normal in infectious mononucleosis[10]. In our laboratory decreased serum muramidase activity was observed in a patient with mononucleosis consisting of large mononuclear cells with basophilic cytoplasm (Downey's type III[21]). The activity returned gradually to normal with the disappearance of these cells.

It is thus apparent that the high serum muramidase level in patients with monocytic leukemia is a remarkable finding among hematologic diseases. This finding is considered as useful for the diagnosis and classification of monocytic leukemia.

Significantly highly increased muramidasuria was demonstrated in all cases of monocytic leukemia before treatment. Such high excretion was never found in other leukemias, including paramyeloblastic leukemia. There have been few reports on muramidasuria in hematologic disorders other than leukemia. Snapper[22] reported that muramidasuria developed occasionally in sarcoidosis

but a clear-cut positive test was found only after concentration of the urine. These findings are in agreement with the result of the present study. Therefore, the determination of urinary muramidase levels may serve as an important indicator for classification of monocytic leukemia from other types of leukemia.

Various degrees of muramidasuria, however, have been reported in patients with renal disorders[23)-25)] and a decreased proximal tubular reabsorption of muramidase is considered the pathogenesis of muramidasuria[23)-27)]. Therefore, the complication of renal dysfunction should be taken into consideration when urinary muramidase level is determined in patients with leukemia.

There have been only a few reports[11)12)] on serial measurements of serum and urinary muramidase in patients with monocytic leukemia. Perillie[11)] suggested a possibility of usefulness of serial measurements of serum muramidase. Wiernik[12)] supported this presumption by some clinical observation. As the result of the present study, in each patient with monocytic leukemia serum and urinary muramidase levels showed a good correlation with clinical status, peripheral leukocyte counts and bone marrow findings and revealed some information. High serum and urinary muramidase activity was apparent before treatment in patients with monocytic leukemia, although the initial muramidase levels in urine, were not so elevated. This reason may be because peripheral leukocytes and bone marrow leukemic cells did not yet reach the worst status. Therefore, it is better for more precise information to measure urinary muramidase levels everyday, because in most cases the levels were elevated progressively and reached the maximum within several days after the initial determinations. After remission, all patients had normal muramidase levels in serum and no excretion in urine. It was impossible to obtain a suggestion whether measurement of this enzyme helps to detect latent leukemia.

Finch[11)] and Wiernik[12)] reported that a sudden increased serum level in a patient in remission preceded by several days the development of overt evidence of hematologic relapse. In the present study, however, serum levels were not elevated before the evidence of marrow relapse in all cases. This may be more reasonable for the inference that serum muramidase is derived from the degradation of leukemic cells[18)28)29)], if a latent focus does not exist. These evidences suggest that serum muramidase level may not be useful to know a relapse in advance. However, serial observation revealed that serum and urinary muramidase levels reflected closely the changes in the clinical course. This fluctuation was a good indicator for the practical management and intensive therapy.

Serum and urinary muramidase assay proved to be helpful for the differential diagnosis between the early stage of monocytic leukemia and reactive monocytosis. As described in case 6, complete leukemic feature did not appear before his late stage, that is, monocytosis was remarkable but monoblasts were only few in the peripheral blood and bone marrow. Other cellularities were almost normal. Moreover, he had a lesion of lung tuberculosis and was

operated for stomach cancer which could cause monocytosis. In this case the critical diagnosis was immediate need to choose what kind of therapy should be given. Elevation of the initial serum and urinary muramidase levels, followed by the progressive increase, was helpful for diagnosis.

Earlier reports[11)18)30)] showing a close relationship between monocyte count and serum muramidase level indicate that monocyte series may be a main source of the elevated serum muramidase levels in monocytic leukemia. However, the higher muramidase content in neutrophils than in monocytes[30)31)32)] and the results in our laboratory that no marked elevation of serum and urinary muramidase was observed in a patient with chronic myelocytic leukemia who had received splenic irradiation with resultant decrease in peripheral leukocyte counts, may necessitate another postulation as possible mechanisms for increased muramidase quantity in monocytic leukemia. Exudative capability of mononuclear cells in patients with monocytic leukemia, followed by transformation into macrophages in tissues with marked increase in muramidase activity[33)] may contribute to the production of large quantities of muramidase in these patients. Anyway, serum and urinary muramidase activity will give important information for diagnosis and prognosis of leukemics.

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