Serum Lysozyme Pattern and Hematological Changes in the Host During Growth of a Transplantable Murine Lymphoma

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ABSTRACT

The serum lysozyme concentrations were determined in the host mice bearing a transplantable ascitic lymphoma during growth of the tumour. Simultaneously, the hematological changes were also recorded. These observations were made at weekly intervals till the animals survived and were made at weekly intervals till the animals survived and were compared with their normal counterparts. It has been shown that there is a progressive increase in the concentration of lysozyme along with the growth of the tumour cells within the peritoneal cavity which reaches a peak on the third week following transplantation of tumour cells and then falls off. The concentration of the enzyme, however, never reaches the normal values observed. This shows a parallelism with the growth pattern of the tumour in general. Total and differential counts of white blood cells revealed a marked rise in the total leukocyte counts and a reversal of the lymphoid-myeloid ratio during the growth of the tumour in the host.

INTRODUCTION

Lysozyme, one of the proteins shown to be associated with certain cancers, is present in extraordinary amounts in urine of individuals with monocytic or monomyelocytic leukaemia (1). Serum lysozyme estimation is a simple, convenient and useful laboratory investigation and a review of literature shows that this enzyme has been implicated as an etiological factor in various disorders and credited with being a prognostic indicator in acute myeloid leukaemia (2).

It seemed worthwhile to concentrate our attention on lysozyme as an expression of cell function as well, rather than merely as an etiological factor. In our previous study involving determination of serum lysozyme levels in a variety of tumour bearing mice we noted an increase in lysozyme con-

centration which seemed to be more of a host-reaction towards neoplastic cell proliferation irrespective of the tumour type and associated factors rather than of any diagnostic significance, at least in mice*. This observation led us to follow the changes in serum lysozyme levels during development and growth of tumours within the host. The study attempts towards an understanding of the role of this enzyme in malignancy and the possible correlationship between tumour growth, peripheral leukocyte count and enzyme level. The present report furnishes observation on Dalton's lymphoma — a transplantable ascitic tumour.

MATERIALS AND METHODS

Dalton's lymphoma, originally a spontaneous tumour of the thymus of DBA/2 mouse (3) is now being maintained in ascitic from by serial transplantation in Swiss mice.

Body weight, tumour cell count, blood cell count and serum lysozyme levels were recorded before and during the growth of the tumour till the animals survived.

Serum lysozyme concentration were determined by the lysoplate method of Osserman and Lawlor (1) using human lysozyme as standard.

RESULTS AND DISCUSSION

Dalton's ascitic lymphoma is a fast growing tumour and the tumour cells proliferate in the host peritoneal cavity producing lethality around the fifth week following tumour transplantation. The increase in body weight is however mainly due to the formation of ascitic fluid and hence it may be noted that even after a reduction in the number of viable cells during the fourth and fifth week of tumour growth, the steady increase in body weight remained unaltered.

It is apparent from the table that there is a direct relationship between the lysozyme level and the tumour cell count both of which show an upward trend from the zero day onward reaching a peak at the fourteenth day of tumour development, after which they fall off gradually. The lowest lysozyme concentration recorded just prior to the death of the tumour bearing host is however far above the normal value.

So far as the leukocyte counts are concerned there seems to be an increase in the total count following an initial depression at the first week of tumour transplantation. The percentage of eosinophil and monocyte before or during the growth of the ascitic tumour remains more or less un-

* Das, S., Basu, A., Mitra, A. Chatterjee, A. and Mitra, S.: (unpublished data).

Table 1. *Changes in Serum Lysozyme Concentration and Hemogram of Host Mice at Weekly Intervals During Growth of Dalton's Ascitic Lymphoma.* (All Figures Indicate Average of 6 Mice of Each Group)

Days Following Tumour Transplantation	Body Weight in gms.	Tumour Cell Count Cells/ml	Blood Cell Count/cmm Differential Count of WBC in Percentage	Serum Lysozyme μg/ml
0 Day	19 ±1.67	0	RBC: 500,0000 WBC: 90,000 N-20%, L-77%, E-1%, M-2%	81.09 ±12.98
7 Days	22.33±2.57	81,000	RBC: 550,0000 WBC: 6,000 N-35%, L-61%, E-1%, M-3%	134.16 ±50.93
14 Days	30 ±0.87	1,40,000	RBC: 350,0000 WBC: 20,000 N-70%, L-27%, E-1%, M-2%	263.14 ±99.29
21 Days	35.83±2.40	1,25,000	RBC: 320,0000 WBC: 26,000 N-75%, L-23%, E-1%, M-1%	205.4 ±39.49
28 Days	38 ±6.63	85,000	RBC: 300,0000 WBC: 28,000 N-85%, L-12%, E-1%, M-2%	143.62 ±39.22
35 Days	44.8 ±0.56	84,000	RBC: 330,0000 WBC: 50,000 N-85%, L-11%, E-1%, M-3%	141 ±20

N-Neutrophil, L-Lymphocyte, E-Eosinophil, M-Monocyte.

changed. While a gradual fall in the lymphocytic counts were noted, the percentage of neutrophil increased considerably during the period of observation. It is interesting to note the appearance of various forms of abnormal and abberant neutrophils during the third, fourth and fifth week of tumour development. Neutrophils with excessive nuclear lobulation were noted in large numbers which are indication of abnormal mitotic activity in these cells. Also mature neutrophils showing unusual nuclear appendages as well as Dohle bodies were found in many cases.

That tumour growth was paralleled by a marked rise in total leukocytes and also induced a reversal of the lymphoidmyeloid ratio have already been reported by many workers (4~7). Delmonte *et al.* (6) observed abberant granulocytic forms in CE, BALB/C and F_1 hybrid strains of mice bearing transplantable mammary cancer and also reported that the peripheral granu-

locyte level was directly proportional to the volume of viable tumour tissue present. While earlier workers believed in the possibility that tumour-associated microorganisms induced the leukemoid reaction (4), such a possibility was ruled out by thers (5～6). It was believed that bacterial pathogenesis was not related to tumour-associated leukaemoid reaction, but suggested that viral-pathogenesis may have some role in the reaction (6). Ray and Guhathakurta (8) also noted progressive neutrophilia and observed an increase in granulocytes and a reversal of lymphoid-myeloid ratio in murine hosts bearing a variety of transplantable tumours viz. Sarcoma 180, Fibrosarcomas, Ehrlich's Carcinoma and L1210.

Caovsky *et al.* (9) had reported of a correlation between the absolute monocytic count in the blood and the lysozyme levels in human leukaemic patients. It was shown that high monocytic counts corresponded to high lysozyme concentration and also that serum lysozyme fell with the remission of the disease and again rose during relapse. Monocytic counts followed the same trend. Our results, however, differ from this observation. We found a similar type of correlation but between neutrophils and lysozyme concentration and upto a certain stage of tumour growth — i. e. upto the third week of development of the ascitic tumour. However, while this increase in neutrophil percentage continued till death the lysozyme level in the serum fell off consistently with the fall in the viable tumour cell count within the peritoneum.

Although a close parallelism could be drawn between the serum lysozyme level, growth pattern of the tumour cell and, to a certain extent, with the neutrophil percentage in circulation, it is rather difficult to suggest a causal relationship between them.

Taking into account the fact that lysozyme concentration has been shown to be related to induction and development of neoplasm and that the enzyme has been considered a natural immunity factor (10), the increased lysozyme levels observed in the present study may be attributed to the growth of the Dalton's lymphoma cells in the host. On the other hand, since granulocytes and monocytes have been indicated as the primary source of lysozyme in mono-myelocytic leukaemia (1, 11), the present enhancement of lysozyme levels could be due to the increase in neutrophils in the blood. However, at the present state of our knowledge it is difficult to come to a satisfactory conclusion regarding the source of the enzyme, the exact nature of the reaction which produced the neutrophilia and the role of either of these in neoplasia, and hence further exploration along these lines are necessary.

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