β -glucuronidase activity of lymph node imprints from malignant lymphomas and chronic lymphocytic leukaemia

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SUMMARY β -Glucuronidase activity was semiquantitatively estimated in the cells of lymph node (LN) imprints from patients with Hodgkin's disease (HD), diffuse non-Hodgkin's lymphomas, chronic lymphocytic leukaemia (CLL), normal lymph nodes, and benign lymphadenopathies. In addition, in some of these cases β -glucuronidase activity was semiquantitatively determined in peripheral blood smear lymphocytes. The β -glucuronidase score (β GS) was very low in the cells of the LN imprints from patients with diffuse non-Hodgkin's lymphomas. The LN lymphocytes of HD had a normal β GS independently of the histological subtype of the disease, while in the LN imprint of CLL the enzyme activity was low, normal, or high. The β GS of the lymphocytes in LN imprints of normal controls and HD were in general significantly lower compared with the lymphocytes of the peripheral blood smears in the same cases. The relation of our findings to the B and T cell origin of malignant lymphomas and chronic lymphocytic leukaemia is discussed.

β -Glucuronidase is a hydrolytic lysosomal enzyme present in normal lymphocytes (Lorbacher *et al.*, 1967; Yam and Mitus, 1968). Its activity has been semiquantitatively estimated in normal circulating lymphocytes and various degrees of positivity have been found (Yam and Mitus, 1968; Flandrin and Daniel, 1974). Peripheral blood lymphocytes from patients with non-Hodgkin's lymphomas and patients with chronic lymphocytic leukaemia (CLL) had a lower β -glucuronidase activity compared with lymphocytes from normal subjects and patients with Hodgkin's disease (HD) (Anlyan *et al.*, 1950; Follette *et al.*, 1952; Yam and Mitus, 1968; Brittinger *et al.*, 1970; Douglas *et al.*, 1973; Flandrin and Daniel, 1974; Westerhausen, 1973; Woessner *et al.*, 1974). Crowder and White (1968), however, found normal β -glucuronidase activity in CLL lymphocytes. These conflicting results and the lack of a semi-quantitative study of β -glucuronidase activity in lymph node (LN) imprints in lymphoproliferative disorders prompted us to investigate (1) whether the activity of this enzyme in cases of malignant lymphocytic proliferation differs from that in benign lymphocytic proliferation, (2) whether it differs in the LN imprint cells and the peripheral blood

lymphocytes in the same cases, and (3) whether it is related to the B or T cell origin of neoplastic lymphoid malignancies.

Patients and methods

A total of 64 patients admitted to the First Department of Internal Medicine, Athens University, during the 12 months June 1973 – May 1974 were subjected to diagnostic LN biopsy. Out of these 64 patients 49 were admitted to the study. Mesenteric lymph nodes from an additional 14 patients undergoing abdominal surgery for non-malignant and non-acute inflammatory disorders were used as controls. All 63 LN specimens were obtained in the fresh state and numerous touch imprints were made from each specimen. The lymph nodes were then fixed in buffered formalin solution and sections cut at 5 μ m and stained with haematoxylin and eosin. Imprints were stained with May-Grünwald-Giemsa for morphological evaluation of the cell type. Blood smears were obtained at the time of LN biopsy from three patients with diffuse lymphocytic, poorly differentiated malignant lymphoma (DLPD); five patients with diffuse lymphocytic, well differentiated malignant lymphoma (DLWD); four patients with HD; five patients with CLL; and 10

Table Data on the cases of 49 patients with malignant lymphoproliferative disease and 14 persons with no malignancy (controls)

Histological diagnosis	No. of cases	Mean age (range)	Sex	
			M	F
Controls	14	53 (32-68)	6	8
Non-specific reactive follicular hyperplasia	8	43 (12-59)	4	4
Tuberculosis	3	19, 58, 60	2	1
Sarcoidosis	3	45, 60, 68	1	2
Leishmaniasis (<i>L. Donovanii</i>)	2	19, 31	1	1
Malignant lymphomas*				
Hodgkin's disease	10	30 (16-65)	5	5
Non-Hodgkin's lymphomas				
Diffuse lymphocytic, well differentiated	7	66 (38-72)	4	3
Diffuse lymphocytic, poorly differentiated	7	53 (17-80)	4	3
Diffuse histiocytic	2	58, 72	1	1
Chronic lymphocytic leukaemia†	7	62 (51-82)	6	1

*Three cases of lymphocytic predominance type, six cases of nodular sclerosis type, one case of mixed cell type.

†Absolute lymphocytes ranged from 10 000 to 130 000/mm³. All cases with significant generalised lymphadenopathy and spleno or hepatomegaly, or both.

controls. The age, sex, and histological diagnosis of all the patients studied are shown in the Table. Cases of DLWD were distinguished from those of CLL and Waldenström's macroglobulinaemia by the haematological and immunoelectrophoretic findings (Pangalis *et al.*, 1977b). The diagnosis of tuberculosis was confirmed by culture studies, while the diagnosis of sarcoidosis was supported by clinical and laboratory studies.

CYTOCHEMICAL STUDY

β -Glucuronidase activity was measured by the cytochemical method described by Hayashi *et al.* (1964) and Lorbacher *et al.* (1967). One hundred peripheral blood lymphocytes were examined and graded from 0 to 4 $\pm$ according to the degree of reaction positivity after counterstaining with methyl green (Yam and Mitus, 1968). In the LN imprints, however, 400 cells were evaluated. In normal LN, benign lymphadenopathies and Hodgkin's disease this evaluation was limited to lymphocytes. In the LN imprints from non-Hodgkin's lymphomas and CLL all cells were available because from the tissue sections the number of apparently normal lymphocytes was either negligible or, in DLWD and CLL, could not be separated morphologically from the neoplastic cells.

The cells of the imprints were counterstained with methyl-green, which enabled the β -glucuronidase positivity to be measured concomitantly with the morphological identification of the individual cells.

Results

The end product of β -glucuronidase reaction was

easily recognised as discrete small reddish granules in the cytoplasm of the cells. The interpretations of reaction positivity were the same both before and after methyl-green counterstaining.

β -GLUCURONIDASE ACTIVITY IN LN IMPRINTS

Controls and benign lymphadenopathies

The lymphocytes of the normal mesenteric LN had an average β GS of 60.5 ± 21.8 (range 3-93) and an average of 48% positive cells (range 30-75%). The β GS of the lymphocytes from the non-specific reactive LN as well as from the LN of tuberculosis, sarcoidosis, and one case of leishmaniasis was within the range of the control lymphocytes (Fig. 1). The only exception was another case of leishmaniasis which had high β GS (173) and 82% of positive cells.

Non-Hodgkin's lymphomas (Rappaport, 1966)

The LN imprints from patients with DLWD, DLRD, and diffuse histiocytic (DH) lymphoma had a very low β GS and a low proportion of β -glucuronidase positive cells (Fig. 1). In only one case of DLRD were both the GS and the percentage of positive cells in the upper limits of normal (75 and 52 respectively).

Hodgkin's disease

In all cases the LN imprint lymphocytes showed a normal β GS and percentage of positive cells regardless of the histological type or the disease (Lukes *et al.*, 1966), including three cases of HD of the predominantly lymphocytic type (β GS 60-89). (Fig. 1).

Fig. 1 β -Glucuronidase activity of LN imprint cells in various benign and malignant lymphadenopathies. NC = controls. RFH = reactive follicular hyperplasia. TBC = tuberculosis. SAR = sarcoidosis. LNS = leishmaniasis. HD = Hodgkin's disease. DLWD = diffuse lymphocytic, well differentiated. DLPD = diffuse lymphocytic, poorly differentiated. DH = diffuse histiocytic. CLL = chronic lymphocytic leukaemia.

Chronic lymphocytic leukaemia

Although the average β GS (59) and the average percentage of positive lymphocytes (49) in the LN imprints of CLL were found to be within normal limits, three patients had a very low β GS while four had a β GS in the upper limits of normal (Fig. 1).

β -GLUCURONIDASE ACTIVITY IN PERIPHERAL BLOOD SMEAR LYMPHOCYTES

Controls

The average β GS of the peripheral blood smear control lymphocytes was 195.5 ± 21.3 (range 164-224) and the average of positive cells 87.4% (Fig. 2).

Non-Hodgkin's lymphomas

The β GS and percentage of positive lymphocytes were both within normal limits in patients with DLPD, while in patients with DLWD the average β GS and percentage of positive lymphocytes were

lower (81.4 and 46.0%) compared with control lymphocytes (Fig. 2). No neoplastic cells were identifiable in the circulation in these cases.

Hodgkin's disease

The β GS and the percentage of positive peripheral blood smear lymphocytes in HD were in the upper limits of normal (212 ± 42.0 and 88.5% respectively).

Chronic lymphocytic leukaemia

The circulating lymphocytes in four of the five patients with CLL had a lower β GS than the controls, while the β GS (178) and the proportion of positive cells (66%) of the fifth patient were within normal limits (Fig. 2).

Discussion

Significant differences in acid phosphatase and β -glucuronidase activities in B- and T-lymphocyte/haematopoietic malignancies have been found by immunological and cytoenzymological techniques (Catovsky *et al.*, 1974a; Brouet *et al.*, 1975;

Fig. 2 β -Glucuronidase activity of peripheral blood lymphocytes in various benign and malignant lymphadenopathies.

Catovsky, 1975; Ritter *et al.*, 1975; Stein *et al.*, 1976; Wehinger and Möbius, 1976). Thus a high β -glucuronidase activity has been found in T-cell CLL and a low enzyme activity in B-cell CLL (Brouet *et al.*, 1975). In addition, Sezary's cells, known to be T-cell in origin, were also found to contain an increased β -glucuronidase activity (Flandrin and Daniel, 1974). Tamaoki and Essner (1969) reported positive acid phosphatase and β -glucuronidase reactions in the T-lymphocyte zone in LN and spleen using frozen sections from man and experimental animals, while the B-lymphocyte zones were negative for both reactions.

Almost all our patients with non-Hodgkin's lymphomas (DLWD, DLPD, DH) had a very low β -glucuronidase activity in the LN imprint cells. Nearly all non-Hodgkin's lymphomas have been classified as B-cell in origin (Stein *et al.*, 1972; Aisenberg and Long, 1975; Lukes and Collins, 1975; Preud'Homme *et al.*, 1975; Braylan *et al.*, 1976). Further studies and correlations with B- or T-cell type on the same material will show whether measurement of β -glucuronidase activity can be used to distinguish these malignant lymphomas. In this context it may be of interest that although no great differences in acid phosphatase and β -glucuronidase activity of normal B and T peripheral blood lymphocytes were found by biochemical and cytochemical methods a very low β -glucuronidase activity was observed in B lymphocytes from tonsil compared with T lymphocytes from tonsil (Pangalis *et al.*, 1977a). The neoplastic cells showed β GS in the upper limits of normal in only one case of DLPD in our series of non-Hodgkin's lymphomas. This was in a 17-year-old boy who presented with mediastinal enlargement and cervical lymphadenopathy. Both the LN sections and imprints showed the picture of lymphoblastic lymphoma (Nathwani *et al.*, 1976) of the convoluted type, a neoplasm that has been reported to be of T-lymphocyte origin (Barcos and Lukes, 1975; Stein *et al.*, 1976).

We found a normal β GS in the HD LN imprint lymphocytes independently of the histological type of the disease. This was significantly different from the β GS of DLWD LN imprint lymphocytes, in which enzyme activity was very low. This difference between HD and DLWD may help in differential diagnosis, particularly because a marked scarcity of Reed-Sternberg cells in HD of the predominantly lymphocytic type may result in an erroneous diagnosis of DLWD (Berard and Dorfman, 1974).

In three out of our seven patients with CLL the LN imprint lymphocytes had a low β GS while in the remaining four the enzyme activity was in the upper limits of normal. Platt and Platt (1971) reported a high β -glucuronidase activity in LN

imprints of CLL, while Salvidio and Baldini (1965), by a biochemical method, found a low β -glucuronidase activity in lyophilised human spleens and lymph nodes in cases of CLL. These observations and our findings indicate that in LN lymphocytes in CLL other factors influence β -glucuronidase activity. Possibly an increased residual T-lymphocyte population in some cases results in a normal β GS (Catovsky *et al.*, 1974b; Braylan *et al.*, 1976; Davis, 1976).

Generally our findings on the β GS in the peripheral blood lymphocytes are in agreement with previous reports (Anlyan *et al.*, 1950; Follette *et al.*, 1952; Yam and Mitus, 1968; Douglas *et al.*, 1973; Westerhausen, 1973; Flandrin and Daniel, 1974; Woessner *et al.*, 1974). Our most interesting observation was that the β GS was three times higher in the peripheral blood lymphocytes than in the LN lymphocytes in the controls and in patients with HD. This may be because of a different type of lymphocyte composition (B and T) or because the functional condition or maturation of the peripheral blood lymphocytes differed from that of the LN lymphocytes. The latter possibility is supported by the fact that we found no significant difference in β -glucuronidase activity in separated normal B and T blood lymphocytes while the β -glucuronidase activity has been found to be very low in B tonsil lymphocytes compared with that in T tonsil lymphocytes (Pangalis *et al.*, 1977a).

Our results are preliminary ones and a study of more cases in each group seems necessary. Nevertheless, they suggest that measurement of β -glucuronidase activity in LN imprint lymphocytes may help to distinguish certain types of malignant lymphomas from each other as well as from benign lymphadenopathies.

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