The Lymphocyte $\beta$-Glucuronidase Activity in Lymphoproliferative Disorders

By LUNG T. YAM AND W. J. MITUS

ALTHOUGH THE LYMPHOCYTES of chronic lymphocytic leukemia morphologically resemble those of healthy subjects, many differences exist between the leukemic and the normal lymphocytes. The lymphocytes from other lymphoproliferative disorders such as lymphosarcoma also differ from normal lymphocytes in glycogen content within the cells and decreased response to phytohemagglutinin (PHA) stimulation. It has been shown that cell growth is associated with increase of lysosomal enzyme activity (acid phosphatase). The decrease of lysosomal enzymes (acid phosphatase, $\beta$-glucuronidase) in the leukemic lymphocytes together with the decrease in responsiveness to PHA stimulation in these cells is of particular interest. The investigation of this relationship may not only provide new data on lymphocytes from various disorders, but also may yield information on the association of lysosomes and cell growth.

The lysosomal enzyme $\beta$-glucuronidase has been shown to exist in human blood. Most of the enzyme activity is in the leukocytes. Anlyan et al. studied the leukocytes $\beta$-glucuronidase by biochemical methods in normal subjects, in patients with infections, leukemia and Hodgkin's disease and noticed that it was consistently low in chronic lymphocytic leukemia. Similar findings had been reported by Follette et al. Because the biochemical methods measure the entire population of leukocytes and not a single type of cell, it is difficult to know whether the changes observed were due to changes in the lymphocytes or in other leukocytes.

Salvidio and Baldini determined the $\beta$-glucuronidase activity from spleens in patients with various disorders and showed that $\beta$-glucuronidase as well as acid phosphatase was low in lymphosarcoma and in chronic lymphocytic leukemia. Since the spleen contains 90 percent or more of lymphocytes, their data probably indicate that the $\beta$-glucuronidase activity is low in the neoplastic lymphocytes. However, the macrophages present in the spleen might still account for the higher value observed in non-lymphomatous spleens.

The purpose of this study is to measure the $\beta$-glucuronidase activity in lymphocytes from normal and leukemic subjects by a semi-quantitative cytochemi-

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Fig. 1.—β-glucuronidase in lymphocytes. (a) Grade 1, (b) Grade 2, (c) Grade 3, and (d) Grade 4. × 1,300.

cal method and to correlate this with the response of the lymphocytes to PHA stimulation.

**Materials and Methods**

A total of 140 subjects were included in this study. Twenty-five of these were healthy controls. An additional five had illnesses other than hematologic or immunologic disorders. The remaining 110 were patients with various hematologic disorders. A total of 171 examinations were made on these 140 individuals.

β-glucuronidase was demonstrated cytochemically by the method of Lorbacher et al. Naphthol-AS-BI-β-D-glucuronide was the substrate and hexazonium pararosanilin was the coupler. Fresh smears were used within three hours after preparation. After fixation with cold formalin-methanol (7:3 at 4–10°C) for 45 seconds, the smears were rinsed with distilled water for one minute, dried at room temperature for 30 minutes and kept at 0°C for one hour. After the freezing procedure, the smears were allowed to thaw. They were then incubated in a substrate—hexazonium pararosanilin solution at pH 5.2 in a water bath at 39°C for one hour. They were rinsed with distilled water, counterstained with one percent methyl green at pH 4.2 for one minute and mounted with synthetic resin.

One hundred lymphocytes were examined and graded on a 0 to 4 scale (Figure 1). Zero indicated no activity: (1) a single large granule or less than 5 small granules; (2) 5–10 small granules or one large granule and 2–5 small granules; (3) 10–15 granules; (4) more than 15. The total was expressed as a score per 100 lymphocytes.

Lymphocyte acid phosphatase activity was studied by the histochemical method of Barka and Anderson with some modifications. The substrate solution (pH 5) contained naphthol AS-BI phosphate and hexazonium pararosanilin. After the smears were fixed with a cold methanol—10 percent formalin solution (3:7) for one minute, they were processed as described for β-glucuronidase (with the exception of substrate). The lymphocytes were also scored in a similar scale as that for β-glucuronidase.
LYMPHOCYTE BETA GLUCURONIDASE ACTIVITY
IN HEALTH AND DISEASE

Fig. 2.—Lymphocyte β-glucuronidase activity in various disorders. N = normal, CLL = chronic lymphocytic leukemia, LSA = lymphosarcoma, IM = infectious mononucleosis, BL = benign lymphozytosis, HD = Hodgkin’s disease, ALL = acute lymphocytic leukemia, RCD = reticulum cell disorders, MPD = myeloproliferative disorders, OHD = other hematologic disorders and NHD = nonhematologic disorders.

Out of the 140 subjects included for lymphocyte β-glucuronidase (LβG) study, PHA stimulated lymphocyte cultures were performed on 39. The method of separating and culturing lymphocytes and evaluation of results was that of Yam, Castoldi and Mitus. The PHA responsiveness of the cells, “transformation index,” is expressed as a percentage.
of absolute number of PHA reacting cells per ml. of culture medium at 72 hours to a total number of viable lymphocytes per ml. at zero hour. Normal range of transformation index is between 35 and 72 percent.

**Results**

$\beta$-glucuronidase activity in the lymphocyte appeared as discrete, reddish granules in the cytoplasm. Activity outside the granules was minimal. The scores of lymphocyte $\beta$-glucuronidase (L$\beta$G) activity from healthy subjects and patients are listed in Figure 2. Low scores were found mainly in patients with chronic lymphocytic leukemia, lymphosarcoma and reticulum cell sarcoma. Values higher than normal were noted in nine patients but such cases were too few to allow any conclusions to be drawn.

*Normal.* The average L$\beta$G was 197 ± 14 (range 140–230). The average value for 16 males was 199 ± 14.1 and the average value in nine females was 193 ± 13.8.

*Chronic lymphocytic leukemia (CLL).* Of the 19 patients examined, 13 had values below the normal range. Two of the six patients with scores over 140 had previously been treated and had responded well to therapy. In the other three patients with very high scores, the clinical pictures were unusual. In one patient with a score of 280, the lymphocyte count in the peripheral blood never exceeded 7,000/mm$^3$. His bone marrow showed 50–60 percent lymphocytes and erythroid hypoplasia, but was never completely replaced by lymphocytes. He also had Kaposi’s sarcoma. The other two patients had a rapidly progressive spread of their disease with serous effusions. They both responded well to cytotoxic drugs. One of these two patients was examined periodically. The L$\beta$G scores were 254 initially, 230 when in partial remission, 90 when in relapse, 150 and 160 when again in partial remission. Her transformation indices were 0, 38.65, 1.69, 8.78 and 6.65 respectively. It is interesting to note that in this case the leukemic lymphocytes present during relapse had low enzyme activity whereas the cells present initially showed an abnormally high value.

Lymphocyte $\beta$-glucuronidase activity was evaluated 28 times in these 19 patients. The leukocyte count at the time when the L$\beta$G estimations were made was more than 20,000/mm$^3$ in 17 and less than 20,000/mm$^3$ in the remaining 11. Of the 17 estimations with leukocytes more than 20,000/mm$^3$, 14 had L$\beta$G values less than 140 (mean 100), 3 had L$\beta$G values of 143, 215 and 254 respectively. In the 11 estimations with leukocyte counts less than 20,000/mm$^3$, 9 had normal L$\beta$G values (mean 219) and two had values of 84 and 94 respectively.

*Lymphosarcoma.* In four of the seven patients examined, the L$\beta$G activity was below normal. The other three had values at the lower limit of normal.

*Infectious mononucleosis and benign lymphocytosis.* The L$\beta$G activity in seven patients with infectious mononucleosis was within normal limits. The atypical lymphocytes usually showed activity of moderate degree but some were completely negative. One patient with whooping cough and one with transient lymphocytosis of unknown etiology also had normal L$\beta$G activity.

*Hodgkin’s disease.* All of the nine patients examined were normal. The L$\beta$G activity was essentially unchanged after therapy in two patients studied.
Reticulum cell disorders. Four patients with reticulum cell sarcoma, three with monocytic leukemia (Schilling type), and three with leukemic reticuloendotheliosis were studied. Two of the four patients with reticulum cell sarcoma and two of the three with monocytic leukemia had $L_\beta G$ values less than 140. All three patients with leukemic reticuloendotheliosis had normal values.

Miscellaneous. Included in this group were 61 patients with various hematologic and nonhematologic disorders. The $L_\beta G$ activity was normal in this group except in nine cases. The diagnoses of these nine patients were as follows: acute myelofibrosis 1 (92), acute lymphoblastic leukemia 1 (125), acute granulocyte leukemia 1 (135), acute erythroleukemia 1 (137), anemia and scleroderma 1 (134), aplastic anemia following the use of Chloramphenicol 1 (126), thrombocytopenia and possible lymphoma 1 (124), osteoporosis 1 (137), and one patient with fever and retroperitoneal lymphadenopathy which revealed reactive hyperplasia on histological examination (113).

Further analysis of the types of lymphocytes with various degrees of $\beta$ glucuronidase activity is listed in Figure 3. In normal subjects, one-quarter to one-third of the cells have $L_\beta G$ activity of grade 3 to 4. These cells contributed 50 percent of the $L_\beta G$ scores. In untreated CLL and in treated cases without definite improvement, most of the cells were grades 0, 1 or 2; the cells with grades 3 and 4 constituted only a small part of the total $L_\beta G$ scores. In cases that responded to therapy, the number of cells with stronger enzyme ac-
CORRELATION OF LYMPHOCYTE BETA GLUCURONIDASE ACTIVITY AND RESPONSE OF LYMPHOCYTES TO PHA STIMULATION

Fig. 4.—Correlation of lymphocyte β-glucuronidase activity and response of lymphocytes to phytohemagglutinin stimulation.

Activity (3 and 4) increased. Lymphosarcoma and infectious mononucleosis had patterns between normal and chronic lymphocytic leukemia.

Response to phytohemagglutinin and LβG scores. The correlation of lymphocyte response to PHA and LβG activity in normal subjects and in cases of CLL, infectious mononucleosis, lymphosarcoma and Hodgkin’s disease is shown in Figure 4. In normal subjects and in patients with untreated Hodgkin’s disease, normal LβG scores were associated with normal response to PHA stimulation. In CLL, seven of the eleven cases had low LβG scores together with low transformation indices. In three, the LβG values were normal or high but the transformation indices were low. In one, both the transformation index and LβG
value were within normal limits. After the therapy, the LβG activity and response to PHA stimulation correlated poorly.

In the lymphocyte cultures with or without PHA, the β-glucuronidase activity in cells was not increased. Many of the actively growing blast cells had no stainable β-glucuronidase activity. In cultures containing many dying lymphocytes, the LβG activity was also found to be low.

Lymphocytes from 34 patients (including 8 CLL and 7 lymphosarcoma) were studied for acid phosphatase levels. The results showed close correlation with the findings in the β-glucuronidase study. The only exception was that although PHA stimulated lymphocytes had low β-glucuronidase activity, the acid phosphatase levels were elevated.

**DISCUSSION**

Normal subjects have strong β-glucuronidase activity in their lymphocytes as shown by a cytochemical method. Lymphocytes from patients with CLL and lymphosarcoma show distinctly decreased levels of this enzyme. With a few exceptions, all the subjects with normal proportions of high scoring cells (grades 3, 4 and perhaps some of 2) had normal transformation indices, while those with high proportions of lymphocytes with little enzyme activity (scores 0–1) had abnormally weak responses to PHA stimulation. It is very likely that the lymphocytes with high enzyme activity are those that respond to PHA stimulation and the lymphocytes with little enzyme activity are nonresponders.

Alyan et al. have observed that in several cases of CLL, their abnormally low LβG levels returned to normal following effective therapy. In this study, we have noted that cases with high leukocyte counts generally had low LβG levels; while low cell counts had higher levels. The cases with low cell counts were usually treated and under good control at the time of the study. It is possible that the neoplastic lymphocyte with little β-glucuronidase activity were replaced by cells with higher enzyme activity to give a higher LβG. In addition, normal or nearly normal responses of lymphocytes to PHA stimulation have been reported in cases of CLL with relatively low counts. This finding supports the concept that there are two (or several) populations of lymphocytes in CLL. The correlation between the leukocyte count and LβG level, however, is not invariable. In a study of lymphocyte response to PHA and in the present cytochemical study, two patients with low leukocyte counts had low LβG levels and poor transformation indices. This would indicate that the number of lymphocytes in the blood per se does not necessarily influence the LβG level or the transformation index. It is probable that the proportion of cells with high enzyme activity (PHA reacting cells?) to those of low enzyme activity (PHA nonreacting cells?) is the important factor.

The common association of low lysosomal activity and poor transformation of lymphocytes from CLL is of great interest. Allison and Mallucci have suggested that lysosomes may be involved in some way in cell division. As cell growth and division is involved in the transformation process of the PHA stimulated lymphocytes, it is logical to consider the poor response of the lymphocytes of CLL to PHA stimulation to be due to the low acid phosphatase and
β-glucuronidase. It has been shown biochemically and cytochemically that while acid phosphatase is increased during the transformation of lymphocytes in PHA stimulated cultures, β-glucuronidase activity changes very little, if at all. In PHA stimulated cultures of CLL, the acid phosphatase content is increased in cells that showed no transformation morphologically. It is still possible that the lysosomal enzymes are essential for transformation in the initial stages and that the low enzyme activities in neoplastic lymphocytes are incapable of triggering the transforming process. In two of the three cases of CLL with elevated acid phosphatase and β-glucuronidase activities, the transformation indices were very low. Thus, although a good correlation exists between the lysosomal enzymes and lymphocyte transformation, this is not absolute, indicating that other factors may be involved as well.

There is little doubt that the lysosomal enzyme, acid phosphatase, increases with cell growth; however, it is still not clear whether the increase of lysosomes initiates the cell growth or is a consequence of it. The fact that another lysosomal enzyme, β-glucuronidase, does not increase with cell growth casts some doubt on the lysosome-cell growth theory. Further cytochemical studies of other lysosomal enzymes are necessary in order to help clarify this problem.

SUMMARY

Lymphocyte β-glucuronidase activity was estimated semi-quantitatively by a cytochemical method. Strong enzyme activity was found to be present in the lymphocytes. In patients with chronic lymphocytic leukemia, lymphosarcoma and some cases of reticulum cell sarcoma, the lymphocyte β-glucuronidase activity was low. This was due to the presence of a large number of lymphocytes with low β-glucuronidase activity. The low β-glucuronidase content in the lymphocytes is usually associated with decreased ability of these cells to undergo transformation upon phytohemagglutinin stimulation; however, this association is not an absolute one.

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