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Cell Biology

γ-Glutamyl transpeptidase, a lymphoid cell-surface marker: Relationship to blastogenesis, differentiation, and neoplasia

(mitogens/bone marrow- and thymus-derived lymphocytes/leukemia/lymphoblastic lines/myeloma/glutathione)

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ABSTRACT **γ-Glutamyl transpeptidase, an enzyme that catalyzes γ-glutamyl transfer from γ-glutamyl compounds to amino acid and peptide acceptors, and which is known to be localized in the membranes of many epithelial cells, was found in a variety of lymphoid cells. The lymphoid cell enzyme is located on the cell surface, and exhibits substantially the same substrate specificity as the enzyme found in epithelial cells. Human and rat (but not mouse) lymphocyte γ-glutamyl transpeptidase was stimulated by treatment of the cells with mitogens.**

Normal human peripheral B-cells were more active than T-cells, but the reverse relationship of activities was found in chronic lymphocytic leukemia lymphocytes. Human lymphoblastic lines vary markedly in activity. In general, cell lines with B- and T-characteristics from patients with lymphoproliferative diseases had much lower activities than those of B-cell lines derived from normal subjects. The highest activity found was in a human myeloma line active in synthesis of an immunoglobulin light chain. The data indicate that γ-glutamyl transpeptidase is a surface marker reflecting differentiation in normal and neoplastic cells.

γ-Glutamyl transpeptidase catalyzes the transfer of the γ-glutamyl moiety of glutathione and other γ-glutamyl compounds to a variety of amino acids and peptides. This membrane-bound enzyme is known to be localized in epithelial cells, for example, those of the intestinal mucosa, proximal renal tubules, ciliary body, and choroid plexus (1, 2). Hypotheses concerning the physiological role of this enzyme, such as a function in amino acid transport across epithelial membranes (2–5), have been based largely on information derived from studies on intact animals or organs and on isolated enzymes; studies on free cell systems have not yet been carried out.

The surface properties and metabolism of lymphoid cells have been studied extensively in recent years in relation to their differentiation and normal and malignant transformation. Many of these studies have been done with lymphocyte suspensions cultured *in vitro*. It therefore seemed of interest to study the distribution and activity of γ-glutamyl transpeptidase in normal and neoplastic lymphoid cells undergoing differentiation. The studies reported here demonstrate the presence of γ-glutamyl transpeptidase activity in lymphoid cells. We have found that the enzyme is located on the surface of the cell and have surveyed its activity in a variety of cell types.

EXPERIMENTAL

Materials. Concanavalin A (Con A), twice crystallized, was obtained from Miles-Yeda, Ltd. Galactose oxidase (67 units/mg) was purchased from Worthington, and neuraminidase (from *Vibrio comma*) was obtained from Behringwerke AG, Germany, as a solution containing 500 units/ml. Pokeweed mitogen (PWM) and lipopolysaccharide (LPS) (from *Escherichia coli* 055) were obtained from Grand Island Biological Co. and Difco, respectively. [*Methyl*-^{3}H]Thymidine (2 Ci/mmol) and [*U-glu*-^{14}C]glutathione were products of the New England Nuclear Corp. L-γ-Glutamyl-*p*-nitroanilide, glycylglycine, and the amino acids were from Sigma. *S*-Acetophenone-glutathione was prepared as described (6).

Cell Lines. Human lymphoblastic lines, obtained from sources given in Table 2, were grown in medium RPMI 1640 containing 16% heat-inactivated fetal calf serum, 100 μg/ml of streptomycin, and 100 units of penicillin per ml. Cell cultures were kept in exponential growth phase by addition of fresh culture medium on alternate days. The cells were tested when their viability was over 90%. Murine cell lines and solid mouse tumors were generously provided by Dr. Joel Buxbaum of the Manhattan Veterans Administration Hospital, New York, N.Y.

Lymphocyte Cultures. Human peripheral blood lymphocytes were obtained from normal subjects by the Ficoll-Hypaque method (7). They were further purified from the adhering cells by passing through a nylon-wool column, and the bone marrow-derived (B-) and thymus-derived (T-) cells were separated by the sheep erythrocyte rosetting method (8). The lymphocytes which did and did not form rosettes with sheep erythrocytes are referred to as T-lymphocytes and B-lymphocytes, respectively. Mouse (CBA strain; male, 6–12 weeks of age), rat (Sprague–Dawley; male, 250 g), and guinea-pig (strain 13; male, 250 g) lymphocytes were obtained from spleens and lymph nodes as described (9). The erythrocytes were selectively lysed by incubation in a buffer containing 0.15 M NH_4Cl, 0.017 M Tris·HCl (pH 7.2) at 0° for 10 min. Mouse, rat, and guinea pig lymphocytes (2×10^6 cells per ml) and human peripheral blood lymphocytes (1×10^6 cells per ml) were cultured in RPMI 1640 medium containing 5% fetal calf serum (heat inactivated) and supplemented with penicillin (100 units/ml) and streptomycin (100 μg/ml) at 37° in an atmosphere of 95% air–5% CO_2. In the [^{3}H]thymidine incorporation studies, aliquots (1 ml) of cell cultures were incubated for 2 hr with [^{3}H]thymidine (2.5 μCi) and isotope incorporation into 5% trichloroacetic acid-insoluble material was measured (9).

Mitogenic Stimulation of Cells. Con A, PWM, and LPS were added to the cultures, as indicated, at a final concentration (per ml) of, 2 μg, 10 μl of reconstituted solution, and 20 μg, respectively. Cells were also treated with the following mitogenic agents as follows: cells 50×10^6/ml in phosphate-buffered saline (PBS) were incubated in 1 mM $NaIO_4$ for 30 min at 0°, or with neuraminidase (50 units/ml) and galactose oxidase (1.4 units/ml) for 30 min at 37°, with shaking. The cells were then washed with PBS and cultured as described above.

Determination of γ-Glutamyl Transpeptidase Activity. Transpeptidase activity was determined with L-γ-glutamyl-

Abbreviations: B-lymphocyte, bone marrow-derived lymphocyte; T-lymphocyte, thymus-derived lymphocyte; Con A, concanavalin A; PWM, pokeweed mitogen; LPS, lipopolysaccharide; PBS, phosphate-buffered saline (pH 7.2); NAGO, neuraminidase and galactose oxidase; SA, specific activity.

Table 1. γ-Glutamyl transpeptidase activity in mitogen-induced lymphoblasts[a]

Source	Mitogen	[^{3}H]Thymidine incorporation (cpm)	Activity
Human peripheral blood	None	1,520	560
	Con A	35,400	1,110
	PWM	15,120	820
Rat spleen	None	12,600	270
	Con A	110,000	3,850
	PWM	61,100	2,860
	$NaIO_4$	115,000	1,390
	NAGO	184,000	2,400
Rat lymph node	None	8,160	150
	Con A	137,000	2,740
Guinea pig spleen	None	1,050	470
	Con A	13,600	290
Mouse spleen	None	10,600	220
	Con A	89,700	100
	LPS	26,100	140

[a] [^{3}H]Thymidine incorporation and enzyme activity (toward γ-glutamyl-*p*-nitroanilide and glycylglycine; units/mg of protein) were determined after culturing the cells for 70 hr with or without mitogenic stimulation.

p-nitroanilide or *S*-acetophenone-glutathione and glycylglycine as previously described (6). The cultured cells were washed twice with PBS and suspended in 0.01 M Tris·HCl (pH 8.0) containing 0.15 M NaCl to give a final protein concentration of 1–2 mg/ml. Where indicated, the cells were sonicated with a Bronson Cell Disruptor. The assay solution contained 0.1 ml of cell suspension (or sonicate), 2.5 mM of either L-γ-glutamyl-*p*-nitroanilide or *S*-acetophenone-glutathione, 30 mM glycylglycine (adjusted to pH 8.0 with NaOH), 0.05 M Tris·HCl (pH 8.0), and 0.075 M NaCl (to give a final ionic strength of 0.15). The solutions were incubated at 37° in a water bath equipped with a reciprocating shaker for 10–30 min and then chilled to 0°. The cells were removed by centrifugation and the absorbance of the supernatant was measured at 305 or 410 nm. In assays with cell sonicates, the reactions were terminated by addition of 0.1 ml of glacial acetic acid and the denatured protein was removed by centrifugation. Activity units are nmol of product (*p*-nitroaniline or *S*-acetophenone-cysteinylglycine) formed per hr/mg of protein. Protein was determined by the method of Lowry *et al.* (10). The γ-glutamylcyclotransferase (EC 2.3.2.4) activity of cell sonicates was determined after centrifugation at 100,000 × *g* for 1 hr by measuring the formation of 5-oxo-[^{14}C]proline from L-γ-[^{14}C]glutamyl-L-α-aminobutyrate (11).

RESULTS

γ-Glutamyl Transpeptidase Activity of Mitogen-Stimulated Lymphocytes. Treatment of human peripheral lymphocytes and rat spleen and lymph node lymphocytes with mitogenic agents led to marked increases in transpeptidase activity (Table 1). T-Cell mitogens [Con A, periodate, and neuraminidase and galactose oxidase (NAGO) (12–15)] as well as T- and B-cell mitogen (PWM) (14) produced this effect. It is notable that treatment of rat lymphocytes with periodate under these conditions led to about 90% inactivation of the cell transpeptidase within the brief period of exposure to periodate. Thus, the high activity found 70 hr after treatment with periodate indicates formation of new enzyme. Although mitogenic stimulation of mouse lymphocytes induced DNA synthesis, transpeptidase activity was not increased. Both T-cell (Con A) and B-cell (LPS) mitogens not only failed to increase activity, but led to significantly lower activity. A similar result was found in guinea-pig lymphocytes with Con A. Study of the kinetics of induction of transpeptidase in rat lymphocytes with Con A (Fig. 1) showed an increase in activity at 21 hr at which time DNA synthesis was not significantly enhanced. The activity continued to increase upon incubation up to 3 days. In striking contrast, Con A failed to increase the activity of mouse lymphocytes.

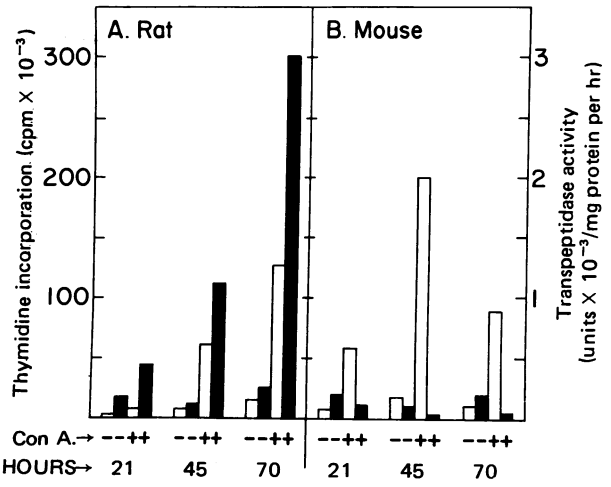

FIG. 1. Effect of Con A on DNA synthesis and γ-glutamyl transpeptidase activity in rat and mouse spleen cells. The cells were cultured in the presence and absence of Con A (2 μg/ml). [^{3}H]Thymidine incorporation (open bars) and transpeptidase activity (with γ-glutamyl-*p*-nitroanilide and glycylglycine; (shaded bars) of the cells was determined.

γ-Glutamyl Transpeptidase Activity of Human Lymphocytes and Lymphoblastic Lines. The activity of a number of human lymphoid cells was surveyed (Table 2). B-Cells from normal peripheral blood have two to three times more activity than do the T-cells. The ratio of activities (B- to T-cells) for the chronic lymphocytic leukemia lymphocytes is much lower than found for the normal B- and T-cell.

Human lymphoblastic lines show much variation in activity (Table 2). The specific activity of lines obtained from normal subjects which possess B-cell characteristics ranged between 994 and 1270. In contrast, the cell lines with B-cell characteristics from patients with acute lymphocytic leukemia and acute myelogenous leukemia had activities ranging from 63 to 370. Low transpeptidase activities were also found in B-cell lines from patients with lymphoproliferatives diseases such as Burkitt and gastrointestinal lymphoma. The cell lines with T-cell characteristics derived from patients with acute lymphocytic leukemia have low activity (range 93–441). Somewhat higher than normal activities were found in B-cells from patients with Bruton's immunodeficiency. The highest activity (line 11, Table 2) interestingly, was found in a line from a patient with multiple myeloma. This cell line is active in synthesis and excretion of λ-light chains of immunoglobulins (16). The high activity of this line was also shown by the histochemical staining technique for transpeptidase activity (17) which qualitatively distinguished this line.

The γ-glutamyl cyclotransferase activities of centrifuged sonicates of cell lines with high and low transpeptidase levels were quite similar. Thus, lines 17 and 11 (Table 2) which had

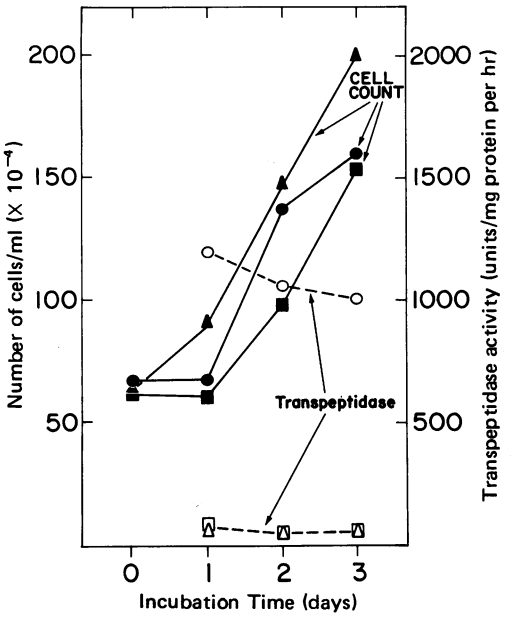

FIG. 2. γ-Glutamyl transpeptidase activity of human lymphoid cell lines at different stages of growth. The cells were cultured and assayed for γ-glutamyl transpeptidase activity with γ-glutamyl-*p*-nitroanilide and glycylglycine. ●,○, Line 3; ▲,△, line 17; and ■,□, line 15. (The cell lines are identified in Table 2.)

transpeptidase specific activities of 93 and 11,300, respectively, showed cyclotransferase activities of 350 and 490, respectively (nmol of 5-oxoproline per hr/mg of protein). The possibility that the lines showing low transpeptidase activity may possess an inhibitor of this enzyme was investigated. Sonicates of line 11 were mixed with those of lines exhibiting low activity (lines 17, 3, and 15) and then assayed. The activity of such mixtures was always additive which makes the possibility of a free inhibitor unlikely.

The transpeptidase activity of several of the human lymphoblastic lines was tested during different phases of growth (Fig. 2). The activities per cell were essentially constant and indicated that the level of the enzyme is a stable characteristic of these lines and not a reflection of the growth phase.

γ-Glutamyl Transpeptidase Activity in Murine Lymphoid Cell Lines and Solid Lymphoid Tumors. The specific activity of murine lymphoid cell lines grown in *in vitro* (maintained by Dr. Joel Buxbaum) were as follows [specific activities (SA) in parentheses]: lines with B-cell characteristics, MPC 11 (SA, 75); MPC 104 (SA, 18); lines 3469, 1748, 471, HPC 76, PU 5R (SA all <10); and line 183 (SA, 33). Solid tumors from which some of the above lines were derived had the following activities: 104 (SA, 39); 3469 (SA, 34); 1748 (SA, 45). The lines with T-cell characteristics had the following activities: ASL1, 81; RADA1, 74; and 129, 115. Thus, the murine lines have relatively low activities. These findings and the observation (Fig. 1; Table 1) that mitogens failed to induce activity in murine lymphoid cells, suggest that the low enzymatic activity of mouse cells is a species characteristic.

γ-Glutamyl Transpeptidase as a Surface Marker in Human Lymphoblastic Lines. That intact cells catalyze the reaction between γ-glutamyl-*p*-nitroanilide and glycylglycine suggests that catalysis occurs at the cell surface. This was further investigated by comparing the activity of sonicated extracts with that of the intact cells (Table 3). Activity was measured with γ-glutamyl-*p*-nitroanilide as well as with *S*-acetophenone-glutathione. The latter, a derivative of glutathione, probably

Table 2. γ-Glutamyl transpeptidase activity of human lymphoblastic lines and peripheral blood lymphocytes

A. Peripheral blood lymphocytes	γ-Glutamyl transpeptidase activity[b] (units/mg of protein)	
Donor[a]	T-cells	B-cells
Normal subjects		
1	234	536
2	240	712
3	121	400
Patients with chronic lymphocytic leukemia		
1	515	87
2	526	159

B. Lymphoblastic lines[c]	γ-Glutamyl transpeptidase activity (units/mg of protein)
1. B, normal subject (CL)	1,200
2. B, normal subject (HH)	1,270
3. B, normal subject (TM)	1,120
4. B, normal subject (RPMI 1788)	994
5. B, normal subject (RPMI 6237)	1,100
6. B, Bruton's immunodeficiency (HT)	2,280
7. B, Bruton's immunodeficiency (J-R)	2,410
8. B, γ3 heavy chain disease (OMM)	283
9. B, Burkitt lymphoma (P1R)	375
10. B, immunodeficiency, thymoma (PO)	577
11. B, multiple myeloma, λ chain (RPMI 8226)	11,300
12. B, gastrointestinal lymphoma (WO)	167
13. B, acute myelogenous leukemia (RPMI 6410)	63
14. B, acute lymphocytic leukemia (SB)	370[d]
15. B, acute lymphocytic leukemia (8392)	105[e]
16. T, acute lymphocytic leukemia (HSB)	441[d]
17. T, acute lymphocytic leukemia (MOLT)	93
18. T, acute lymphocytic leukemia (CEM)	147
19. T, acute lymphocytic leukemia (8402)	142[e]

[a] T- and B-cells were separated from human peripheral blood as described in *Experimental*.

[b] The activity of the cells was determined with γ-glutamyl-*p*-nitroanilide and glycylglycine, and is expressed as nmol of *p*-nitroaniline released / hr per mg of protein.

[c] The sources of the lines were as follows: Lines 1–3 and 14–19, from Dr. Marc E. Weksler (Cornell University Medical College); Lines 6, 7, 10, and 12, from Dr. S. D. Litwin (Cornell University Medical College); Line 8, from Dr. J. Buxbaum; Lines 4, 5, 9, 11, and 13, from Associated Biomedic Systems, Inc., Buffalo, N.Y.

[d] Derived from the same patient.

[e] Derived from the same patient.

does not permeate the cell membrane. In experiments in which two cell lines (lines 3 and 11; Table 2) were incubated with [^{14}C]glutathione (glutamate labeled) there was essentially no uptake of label into these cells. As shown in Table 3, the activity of the intact cells was similar to that of the sonicates. The data, thus, strongly indicate surface localization of the enzyme. The activity of the cells with *S*-acetophenone-glutathione was 70–75% of that with γ-glutamyl-*p*-nitroanilide; similar relative activities were previously observed with purified rat kidney transpeptidase (18).

Table 3. Comparison of the γ-glutamyl transpeptidase activity of intact and sonicated human lymphoblastic cells

Source of the enzyme	Transpeptidase activity[a] With-L-γ-glutamyl-*p*-nitroanilide	Transpeptidase activity[a] With *S*-acetophenone-glutathione
TM[b]		
Intact cells	1090	774
Sonicate	1140	800
RPMI 8226[c]		
Intact cells	8950	7680
Sonicate	9090	7170

[a] The assays were performed in the presence of either 2.5 mM L-γ-glutamyl-*p*-nitroanilide or 2.5 mM *S*-acetophenone-glutathione and 20 mM glycylglycine. The values given are specific activities (nmol/hr per mg of protein).

[b] Human lymphoblastic cell line with B characteristics (from normal subject).

[c] Human lymphoblastic cell line from a patient with multiple myeloma.

Table 4. Activity of intact lymphoid cell γ-glutamyl transpeptidase towards various amino acids and peptides[a]

Acceptor	Specific activity of human lymphoid line (TM)	Specific activity of rat spleen lymphoblasts induced by Con A
None	93 (8)	327 (8)
Glycylglycine	1120 (100)	3850 (100)
L-Alanyl-glycine	645 (58)	1630 (43)
Glycyl-L-alanine	975 (87)	2350 (61)
Glycyl-D-alanine	77 (7)	312 (8)
L-Glutamine	310 (28)	954 (25)
L-Methionine	143 (13)	1050 (27)
Glycine	93 (8)	447 (12)
L-Glutamate	109 (10)	608 (16)
L-Valine	81 (7)	320 (8)
L-Serine	180 (16)	447 (12)
L-Alanine	143 (13)	—
L-Lysine	47 (4)	—
L-Arginine	128 (11)	—
Glycylglycine + L-serine + borate	169 (15)	—

[a] The activity of the cells was determined with 2.5 mM L-γ-glutamyl-*p*-nitroanilide and 20 mM acceptor (specific activity, nmol/hr per mg of protein). Inhibition by L-serine plus sodium borate was studied at a concentration of 2 mM each. The specificity of human lymphoid lines (11 and 4; Table 2) was also studied with similar results; thus, the relative activities with glycylglycine, L-glutamine, and L-methionine were, respectively, 100, 22, and 12; and 100, 33, and 12, respectively. Relative activities are given in parentheses.

Specificity of γ-Glutamyl Transpeptidase of Intact Lymphoid Cells. The specificity of intact human lymphoid lines (lines 3, 11, and 4; Table 2) and rat spleen lymphoblasts induced by Con A was examined using various acceptors; representative data are given in Table 4. The dipeptides, in general, were much better substrates than the amino acids; glycylglycine was most active. The most striking difference between the human and rat lymphoid cells was the relatively higher activity of L-glutamine as compared with other amino acids with the human cell line enzyme. In general, the specificities shown in Table 4 closely resemble those observed previously with purified human (19) and rat kidney (6, 18) γ-glutamyl transpeptidases, respectively. L-Serine, in the presence of borate, strongly inhibited the activity of intact cells; such inhibition occurs with γ-glutamyl transpeptidases from other sources (2, 20).

DISCUSSION

Earlier studies on γ-glutamyl transpeptidase have emphasized its localization in epithelial cells (1, 2). Thus, the present finding of the enzyme in lymphoid cells, which are of mesenchymal origin, indicates that this enzyme enjoys a broader distribution in mammalian cells than previously recognized. It is notable that in both lymphoid and epithelial cells the enzyme is primarily membrane-bound. The ability of lymphoid cells to interact with γ-glutamyl donor substrates that do not enter the cells shows that the active site of the enzyme is on the cell surface.

The transpeptidase activity of human and rat lymphocytes was markedly increased by mitogenic stimulation. T-Mitogens as well as a mixed B- and T-mitogen were active. Of special interest is the finding that mitogenic stimulation of mouse and guinea pig lymphocytes did not increase the activity of these cells. Although the reasons for this are presently unknown, it may be noted that previous studies have shown significant differences in the properties of lymphocytes from different species. Thus, neuraminidase-treated human and rat lymphocytes were stimulated by peanut agglutinin whereas neuraminidase-treated mouse and guinea pig lymphocytes were not (9). In mitogen-stimulated rat lymphocytes, the increase in activity preceded the stimulation of DNA synthesis [a finding in accord with earlier data on protein synthesis in mitogen-stimulated cells (21)], and continued to increase with increased DNA synthesis, but in mouse lymphocytes, mitogen (Con A) did not increase the enzyme activity during blastogenesis. It is notable that because periodate-treatment [which induces blastogenesis (12)] rapidly inactivates lymphocyte transpeptidase, the observed increase in enzyme activity of periodate-treated rat lymphoblasts most probably be ascribed to *de novo* synthesis of enzyme. The low activity of mitogen-induced murine lymphoblasts is also reflected in the relatively low activities found in murine cell lines with either B- or T-cell characteristics.

The transpeptidase activity of normal human B-lymphocytes is two to three times greater than that of the T-lymphocytes. However, B-cells from patients with chronic lymphocytic leukemia exhibited much lower activity than normal B-lymphocytes. This observation supplements previous data which indicate that chronic lymphocytic leukemia cells have impaired surface properties and function (22). Although marked variation was found in the activity of human lymphoid cell lines, it appears that in general, cell lines with B- and T-characteristics derived from patients with various leukemias and other lymphoproliferative diseases had much lower activities than the B-cell lines derived from normal subjects. The possibility must be considered that the cell lines with low activity contain a modified form of the enzyme which is less active.

It is of considerable interest that a line derived from a patient with multiple myeloma, which has structural and functional characteristics of a myeloma cell (16), has a very high activity, i.e., from five to 200 times greater than that of the other human lymphoid cell lines examined. This cell line is highly active in the synthesis and excretion of the light chain of immunoglob-

ulins (λ chains). The possibility that transpeptidase may be involved in an aspect of synthesis and secretion of immunoglobulins should therefore be considered. The enhanced activity of the transpeptidase in mitogen-stimulated lymphoblasts may indicate a relationship of the enzyme to the synthesis and secretion of lymphokinins and immunoglobulins by these cells (23, 24).

The surface localization of γ-glutamyl transpeptidase and the fact that the enzyme may be readily determined should make this enzyme useful as a surface marker for normal and neoplastic lymphoid cells in various stages of differentiation. It could well prove of value in the diagnosis and prognosis of various lymphoproliferative and other diseases of the immune system. The present studies indicate that the enzyme can be detected by the histochemical γ-glutamyl transpeptidase procedure.

It is of interest that the acceptor and donor specificities of human and rat lymphoid cells are similar to those exhibited by the respective kidney enzymes. It will be of interest to compare the structural relationships of the enzymes obtained from these cell types. The availability of lymphoid cells which differ greatly in γ-glutamyl transpeptidase activity and which can be studied in cell suspensions, may offer a convenient experimental system for further studies on the physiological role of the enzyme.

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