BIOCHEMICAL ACTIVITIES OF NINE LYSOSOMAL ENZYMES IN T AND NON-T LYMPHOCYTES

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1. Introduction

It is well known that lysosomes play an important role in physiologic and pathologic events by degradation of materials and by secretion of their contents [1–3]. Lymphocytes contain considerable activities of lysosomal acid hydrolases which can be demonstrated biochemically, but the number of lysosomes which can be detected morphologically is small. The functional significance of lysosomes in lymphocytes remains to be elucidated. It has been suggested that their lysosomes might be involved in cellular events preceding cell transformation and proliferation [5,6], and that lymphocyte subpopulations could be distinguished cytochemically by their differential reactivity of lysosomal enzymes [7–11].

We previously reported characteristic differences in the composition of lysosomal enzymes in human peripheral lymphocytes and granulocytes [4]. In this communication we extend the observation to lymphocyte subpopulations and discuss the possible significance of different patterns of nine lysosomal enzymes in T and non-T lymphocytes.

2. Materials and methods

Heparinized venous blood of 150 ml each was obtained from eight healthy adult volunteers, and the lymphocytes were separated by centrifugation on a sodium metrizoate-ficoll gradient (Lymphoprep; Nyegaard and Co. Norway) [12]. For separation of T and non-T lymphocytes, a method based on the spontaneous rosette formation of human T lymphocytes with sheep erythrocytes (SRBC) was used [13]. An equal volume of mononuclear cell suspension (5×10^6/ml) and 2% SRBC in fetal calf serum was mixed. Mononuclear cells forming SRBC rosettes were then separated from cells not forming rosettes by centrifugation on the gradient as noted above. Unrosetted cells remaining at the interface and rosetted cells sedimenting to the bottom of the gradient were then removed separately; the SRBC in the pellet fraction were disrupted by Tris-buffered ammonium chloride, and the remaining cells were used as T lymphocytes. The interface cells were re-rosetted and centrifuged on a second gradient in order to remove residual rosetting T lymphocytes. Then, monocytes contaminated in unrosetted cell fraction were removed by treatment with carbonyl iron [14]. These cells were used as non-T lymphocytes. Contaminating granulocytes and monocytes were examined by Wright staining and by non-specific esterase staining [15]. T lymphocytes in the non-T fraction and B lymphocytes in the T fraction were re-examined by formation of SRBC rosettes and by a direct immunofluorescence technique using fluorescein-conjugated polyvalent goat anti-human immunoglobulin (Behringwerke AC, F.G.R.).

Afther three washings with phosphate buffered saline, the cell pellet was suspended in 0.1% triton X-100 and disrupted in a Dounce homogenizer. The cell homogenate was then sonicated (9 kHz, 3 min), following centrifugation at $450 \times g$ for 10 min. The supernatant was used as a cell lysate.

The amounts of enzyme activities in cell lysates were determined under optimal conditions, using the 4-methylumbelliferyl compounds (Koch-Light

Labs, Ltd., U.K.). The conditions for measurement of enzyme activities have been reported previously [4]. Aliquots of 100 μl each of substrate solution in 0.2 M acetate buffer and cell lysate were incubated at 37°C. The reaction was terminated by the addition of 3.3 ml of 50 mM glycine–NaOH buffer (pH 10.4) containing 5 mM EDTA. Fluorescence was measured in a Fluorescence spectrophotometer at an excitation wavelength of 365 nm and at an emission wavelength of 450 nm. The activities were on a cellular basis (per 10^7 cells), on a protein basis (per mg protein in lysate) and on a DNA basis (per g DNA in lysate). The protein and DNA in cell lysate were determined by the methods of Lowry et al. [16] and Hinegardner [17], respectively.

3. Results

3.1. *Purity of prepared T and non-T lymphocyte fractions*

The purity of the T lymphocyte fraction was over 97%. In the non-T lymphocyte fraction, contaminating T lymphocytes and monocytes were both below 3%. The contaminating granulocytes in this fraction were 3.6% to 13.0% (mean 5.6%), mainly basophilic granulocytes.

3.2. *Protein and DNA content in cell lysates of T and non-T lymphocytes*

Table 1 shows no difference of DNA content (μg DNA/10^7 cells) between T and non-T lymphocytes. However, the protein content (mg protein/10^7 cells) and protein-DNA ratio in the non-T cells were significantly higher than that in the T cells. The protein content of the non-T lymphocyte lysate prepared in this study was higher than that of the T lymphocyte lysate.

3.3. *Lysosomal enzyme activities in T and non-T lymphocytes*

Table 2 shows the activities of nine lysosomal enzymes in T and non-T lymphocytes. When the activity was expressed on a cellular or a DNA basis (per 10^7 cells or per μg DNA), acid phosphatase and aryl sulphatase activities were significantly higher in non-T lymphocytes than in T lymphocytes ($p < 0.01$). The α-galactosidase, β-galactosidase and α-mannosidase activities in non-T lymphocytes also tended to be higher than in T lymphocytes. The activity of *N*-acetyl-β-glucosaminidase and β-glucuronidase was the same in non-T and in T lymphocytes. Only α-fucosidase showed a higher activity in T lymphocytes than in non-T lymphocytes ($p < 0.05$).

On the other hand, when the activity was expressed on a protein basis, the difference of enzyme activities between T and non-T lymphocytes differed from that expressed on a cellular or DNA basis. This was due to the different protein content between the two cell populations, as shown in table 1. The activity on a protein basis is inadequate to compare the two cell populations.

Table 3 shows the enzyme activities in the unseparated lymphocyte fraction for comparison with those in the lymphocyte subpopulations.

4. Discussion

It has been reported that cytochemical examination of certain lysosomal enzymes provides useful means for differentiating lymphocyte subpopulations. These cytochemical studies indicate that T lymphocytes show a relatively higher lysosomal enzyme activity than other types of lymphocytes (B and null cell populations) [7–11].

Table 1
Protein and DNA contents in T and non-T lymphocytes

	Protein/10^7 cells (mg)	DNA/10^7 cells (μg)	Protein/DNA (mg) (μg)
T-lymphocytes	1.62 ± 0.31[a]	21.35 ± 3.04	0.07 ± 0.02
Non-T lymphocytes	2.26 ± 0.69[b]	20.88 ± 4.56	0.13 ± 0.04[c]

[a] Mean ± 1 S.D.
[b] $p < 0.05$
[c] $p < 0.01$

Table 2
Lysosomal enzyme activities in T and non-T lymphocytes

Enzyme	Lymphocyte	Activity (Mean ± S.D.)		
		pmol hydrolyzed per min per 10^7 cells	pmol hydrolyzed per min per μg DNA	pmol hydrolyzed per min per mg protein
Acid phosphatase	T cell	1129 ± 294	52.8 ± 9.7	709 ± 184
(pH 5.0)	Non-T cell	1642 ± 244	106.6 ± 19.8	791 ± 215
N-Acetyl-β-glucosaminidase	T cell	527 ± 167	25.0 ± 7.1	330 ± 90
(pH 6.0)	Non-T cell	411 ± 175	22.3 ± 5.4	166 ± 51
β-Glucuronidase	T cell	833 ± 170	41.8 ± 6.8	550 ± 79
(pH 3.5)	Non-T cell	650 ± 152	39.5 ± 10.1	304 ± 101
α-Galactosidase	T cell	75 ± 14	3.4 ± 0.6	46 ± 8
(pH 4.5)	Non-T cell	93 ± 18	5.7 ± 2.5	42 ± 12
β-Galactosidase	T cell	167 ± 37	6.9 ± 1.8	111 ± 40
(pH 4.0)	Non-T cell	194 ± 40	12.4 ± 1.4	89 ± 15
α-Mannosidase	T cell	58 ± 16	2.8 ± 0.7	36 ± 6
(pH 4.5)	Non-T cell	85 ± 28	4.2 ± 0.7	33 ± 9
α-Fucosidase	T cell	211 ± 40	10.0 ± 1.6	132 ± 26
(pH 5.5)	Non-T cell	149 ± 46	8.4 ± 1.6	65 ± 23
β-Fucosidase	T cell	20 ± 9	1.0 ± 0.5	13 ± 7
(pH 4.5)	Non-T cell	27 ± 11	1.6 ± 0.5	13 ± 5
Aryl sulphatase	T cell	32 ± 7	1.7 ± 0.6	21 ± 9
(pH 5.0)	Non-T cell	82 ± 46	4.2 ± 1.6	33 ± 13

In contrast to cytochemical findings, the results of our biochemical study indicate that the non-T lymphocytes showed relatively higher activity of lysosomal enzymes than T lymphocytes; acid phosphatase and aryl sulphatase activities were higher in non-T lymphocytes than the other ($p < 0.01$) and only α-fucosidase showed a higher activity in T than in non-T lymphocytes.

Table 3
Lysosomal enzyme activities in unseparated lymphocytes[a]

Enzyme	Activity (Mean ± S.D.) $n = 6$ pmol hydrolyzed per min per 10^7 cells
Acid phosphatase	3667 ± 1548
N-acetyl-β-glucosaminidase	494 ± 173
β-Glucuronidase	859 ± 190
α-Galactosidase	121 ± 37
β-Galactosidase	495 ± 152
α-Mannosidase	54 ± 11
α-Fucosidase	378 ± 116
β-Fucosidase	26 ± 5
Aryl sulphatase	16 ± 5

[a] Lymphocyte fraction with monocytes removed

There are at least two possible explanations for the difference between cytochemical and biochemical activities of lysosomal enzymes in lymphocyte subpopulations. The first is a different differentiation of lysosomes, as an intracellular organelle, in T and non-T lymphocytes, reflecting their cellular functions. Mueller et al. [7] reported that the cytochemical activity might be acquired by T lymphocytes just before, during/or shortly after peripheralization. Horwiz et al. [8] noted that a single intense reaction by acid esterase staining, which is characteristic in T lymphocytes, disappears after mitogen-induced transformation. These findings suggest that the mode of existence of lysosomal enzymes in T lymphocytes changes with their maturation stage or cellular activation by mitogens. In non-T lymphocytes (B and null cells), the bulk of lysosomal enzymes might exist as a mode undetectable by previously reported cytochemical methods.

The second is that the relatively lower biochemical activities of lysosomal enzymes in T lymphocytes

could result from activation by SRBC rosette formation. In this study, non-T lymphocytes were prepared only by removing T lymphocytes and monocytes, in order to minimize unfavourable effects on the cells. It is reasonable that SRBC rosette formation is a kind of stimulation to T lymphocytes. The enzyme activities might be affected by these cell-to-cell interactions. Table 3 shows the activities of the same enzymes in unseparated lymphocytes. The activities of acid phosphatase, β-galactosidase and α-fucosidase in prepared lymphocytes subpopulations were lower than those in unseparated lymphocytes, while *N*-acetyl-β-glucosaminidase, β-glucuronidase, α-mannosidase and β-fucosidase retained almost the same activities. These results may indicate, in part, that selective fluctuation of the enzyme activity in T lymphocytes follows SRBC rosette formation, such as enzyme secretions.

The functional significance of relatively high lysosomal enzyme activities in lymphocytes remains to be elucidated [4]. The possible significance of these enzymes in T and non-T lymphocytes include their roles in degradation of antigenic materials, modulation of immunological substances and cell-to-cell interactions during immunological responses. Paus and Steen [18] reported that α-mannosidase acts as a mitogen in a manner similar to that of mitogenic lectins.

Further studies are needed to clarify the relationship between the function of lymphocytes and lysosomal enzymes.

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