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Binding receptors for α-L-fucosidase in human B-lymphoid cell lines

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An established mechanism for directing newly made acid hydrolases to lysosomes involves acquisition of mannose 6-phosphate residues by the carbohydrate portion of acid hydrolases followed by binding to specific membrane-bound transport receptors and delivery to lysosomes. Two distinct phosphomannosyl receptors (CI-MPR and CD-MPR) have been identified. Alternative mechanisms for trafficking acid hydrolases exist. This report examines means for the possible receptor-mediated intracellular transport of α-L- fucosidase in lymphoid cells. The binding of α-L-fucosidase to intact cells and to total cell membrane preparations, in conjunction with immunoassays of solubilized membrane preparations, revealed the presence of CI-MPR and CD-MPR on human lymphoid and fibroblast cell lines. The mean level of CD-MPR in nine lymphoid cell lines was 7.2-fold greater than CI-MPR. The mean level of CI-MPR in two fibroblast lines was 3.8-fold greater than CD-MPR. The mean content of CI-MPR was 19.5-fold greater in the fibroblasts than in the lymphoid cells. The CD-MPR content of fibroblasts and lymphoid cells was nearly equivalent. Among these cell lines were a fibroblast and a lymphoid line from the same individual. These results indicate that human B-lymphoid cells are deficient in CI-MPR and suggest that modulation of expression of CI-MPR and CD-MPR in lymphoid cells differs from that in fibroblasts, including cell lines with identical genomes. No specific receptor capable of binding α-L-fucosidase independent of mannose 6-phosphate was demonstrable, despite published results that support the existence of a mannose 6-phosphate independent trafficking mechanism in lymphoid cells for this enzyme.

Keywords: α-L-Fucosidase, phosphomannosyl receptors, B-lymphoid cells

The phosphomannosyl recognition system is an important mechanism for directing newly synthesized acid hydrolases from the Golgi complex to lysosomes [1–3]. The acquisition of mannose 6-phosphate residues by acid hydrolases leads to binding of mannose 6-phosphate containing ligands to specific membrane-bound transport receptors (phosphomannosyl receptors) and their delivery to lysosomes. Two distinct phosphomannosyl receptors (CI-MPR and CD-MPR) have been identified [2–9]. CI-MPR has a subunit of $M_r = 270\,000$ and binds ligands independently of divalent cations. CD-MPR is immunologically distinct from CI-MPR, has a subunit of $M_r = 46\,000$, and exhibits enhanced binding of ligands in the presence of divalent cations. Cell types that contain both CI-MPR and CD-MPR or only CD-MPR have been identified [6, 7, 10–12].

Evidence has been presented supporting the existence of alternative pathways for targeting acid hydrolases to lysosomes. Cells from patients with I-cell disease (ICD) or pseudo-Hurler polydystrophy (PHP) are unable to synthesize the mannose 6-phosphate recognition marker because of inherited deficiency of *N*-acetylglucosamine 1-phosphotranferase (UDP-*N*-acetylglucosamine: lysosomal enzyme *N*-acetylglucosaminylphosphotransferase, EC 2.7.8.17) [13, 14]. Consequently, acid hydrolases cannot bind to phosphomannosyl receptors. In fibroblasts, this enzyme deficiency results in an increased secretion of many acid hydrolases and a corresponding intracellular deficiency of these enzymes. However, while extracts of liver, spleen, kidney, brain, and leucocytes from ICD and/or PHP patients lack *N*-acetylglucosamine 1-phosphotransferase activity, they contain nearly normal activities of intracellular acid hydrolases [15–18]. Moreover, residual acid hydrolase activities in ICD fibroblasts are found in lysosomes and *N*-acetyl-β-D-hexosaminidase from ICD fibroblast lysosomes did not contain the mannose 6-phosphate recognition marker [19].

The biochemical expression of α-L-fucosidase (α-L-fucoside fucohydrolase, EC 3.2.1.51) in lymphoid cell culture has been investigated as a convenient experimental system for understanding alternative mechanisms for targeting acid

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hydrolases to lysosomes [20, 21]. It was reported that lymphoid cell cultures from patients with ICD and PHP retained normal levels of intracellular α-L-fucosidase despite deficient *N*-acetylglucosamine 1-phosphotransferase activity and nondetectable synthesis of mannose 6-phosphate residues on ICD α-L-fucosidase [18, 20, 21]. This suggests a second, phosphomannosyl independent mechanism for intracellular retention of α-L-fucosidase in lymphoid cells. Additional support for this proposal comes from other observations. The carbohydrate component of the majority of mature, processed, intracellular α-L-fucosidase from lymphoid cells of a healthy individual and of all of the mature, intracellular α-L-fucosidase from ICD and PHP lymphoid cells was resistant to endo-*N*-acetyl-β-D-glucosaminidase H (endo-H) [20–23]. Furthermore, the phosphomannosyl recognition marker of acid hydrolases is putatively located on endo-H sensitive carbohydrate chains [2, 3, 24]. Thus, intracellular trafficking of α-L-fucosidase containing endo-H resistant carbohydrate chains may occur by a phosphomannosyl independent pathway.

Experiments involving the binding and uptake of acid hydrolases into fibroblasts were instrumental in the discovery of the phosphomannosyl recognition system for the translocation of acid hydrolases to lysosomes via CI-MPR [25–29]. Additionally, experiments involving the binding of acid hydrolases to cell membrane preparations aided in the recognition of CD-MPR [6, 10, 11]. Each receptor is believed to function in the intracellular translocation of newly made acid hydrolases. However, only CI-MPR functions in the binding and internalization of exogenous acid hydrolases [30].

The objective of this report is to identify possible receptors for trafficking of α-L-fucosidase in lymphoid cells of healthy individuals and of ICD and PHP patients by investigating the binding properties of lymphoid cell α-L-fucosidase to intact lymphoid cells and to membrane preparations.

Materials and methods

Materials

Cell culture medium components were purchased from GIBCO Laboratories, Grand Island, NY, USA except for Nutridoma-HU which was obtained from Boehringer Mannheim Biochemicals, Indianapolis, IN, USA. Triton X-100, saponin, aprotinin, amino acids, simple saccharides, yeast mannan, and fucoidan were purchased from Sigma Chemical Co., St. Louis, MO, USA. Phosphomannan (pentamannose monophosphate) from *Hansenula holstii* was the generous gift of Dr. M. E. Slodki, United States Department of Agriculture, Peoria, IL. The reagent for total protein assays was from Bio-Rad, Richmond, CA, USA.

Cell lines

The B-lymphoid cell lines B142, 204, 212, 219, and 222 were established from peripheral blood of healthy individuals [22], JTL and GM from unrelated fucosidosis patients [31, 32], 1141 from an ICD patient [18], and 1818 from a PHP patient [18]. The fibroblast cell line, WI38, was derived from normal embryonic lung [33] and the fibroblast line, JTF, from skin biopsy of a fucosidosis patient [31]. JTL and JTF were derived from the same individual. Fucosidosis is an inherited metabolic disease due to deficiency of α-L-fucosidase activity [34]. The fucosidosis cell lines were devoid of α-L-fucosidase activity [22, 31, 32, 35]. GM (GM1025) was obtained from the Mutant Cell Repository, Camden, NJ, USA, and WI38 from American Type Culture Collection, Rockville, MD, USA. Procedures for routinely growing all cell lines have been published [22, 31].

Binding of α-L-fucosidase to intact cells

Lymphoid cells (JTL or GM), growing exponentially under routine conditions in 75 cm^2 flasks, were harvested at ambient temperature by centrifugation at 250 × *g* for 10 min and washed twice with phosphate-buffered saline. Cells were suspended at a concentration of 10^6 cells ml^{-1} in 4 ml of incubation medium supplemented with α-L-fucosidase (350 units/ml) purified from B142 cells. Control incubations either lacked α-L-fucosidase or cells. Incubation medium was composed of RPMI 1640 medium supplemented with 20 mM hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, 2 mM L-glutamine, 1% PSN (penicillin, streptomycin, neomycin) antibiotic mixture, and 1% Nutridoma HU. Cultures were incubated at 37 °C in a 5% CO_2 atmosphere for 24 h and were harvested at 4 °C by centrifugation at 250 × *g* for 10 min. The medium was aspirated and saved for assay of α-L-fucosidase activity. Cell pellets were washed twice at 4 °C with phosphate-buffered saline, extracted with 0.2 ml of 0.1% (v/v) Triton X-100, and assayed for α-L-fucosidase activity and total protein. The difference in cell associated α-L-fucosidase activity between experimental and control incubations is a sensitive measurement of binding exogenous enzyme to cells, since JTL and GM cells lack endogenous α-L-fucosidase activity.

Experiments with JTF fibroblasts were conducted with confluent monolayers of cells in 25 cm^2 flasks. The monolayers were rinsed twice at ambient temperature with phosphate-buffered saline. 4 ml of incubation medium supplemented with α-L-fucosidase (350 units/ml) from B142 cells were added to the cultures. The incubation conditions and controls were as described above. Medium was aspirated and saved for assay of α-L-fucosidase activity. Monolayers were harvested at 4 °C by rinsing twice with phosphate-buffered saline. The cells were removed with a rubber policeman and pelleted at 250 × *g* for 10 min. The cell pellets were extracted as described above and assayed for α-L-fucosidase activity and total protein.

Structural requirements for binding of α-L-fucosidase to cells were evaluated by inclusion of various carbohydrates or amino acids in the incubation medium and by comparing

binding of α-L-fucosidase to cells obtained in the presence of these compounds with binding in their absence.

Binding of α-L-fucosidase to purified cell membranes

The purification of total cell membranes and membrane binding assays were conducted as described [6]. Routinely, the binding mixture contained (in a final volume of 0.15 ml) 100 μg of membrane protein, 285 units of purified α-L-fucosidase, 50 mM hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, 5 mM sodium-β-D-glycerophosphate, 0.5% saponin, 0.02 units of aprotinin, and 10 mM $MnCl_2$. The mixtures were incubated at ice temperature for 1 h. The membranes were pelleted at 40 000 × *g* for 0.5 h at 4 °C in incubation buffer. The washing procedure was repeated three times and the washed membranes assayed for α-L-fucosidase activity. Controls lacking exogenously added α-L-fucosidase displayed negligible α-L-fucosidase activity.

Immunoassay of CI-MPR and CD-MPR

The phosphomannosyl receptors were extracted [8] from cell membranes and quantified [9] as previously described. Purified CI-MPR, CD-MPR, and β-D-galactosidase as well as rabbit antisera to CI-MPR and CD-MPR used in the immunoassay procedure were generously supplied by George W. Jourdian, University of Michigan, Ann Arbor, MI, USA. Briefly, the immunoassay involves 1, immobilization of each antiserum to separate wells of a microtiter plate; 2, binding of receptor (known standards or unknown) to immobilized antiserum; 3, binding of β-D-galactosidase to receptor; and 4, measurement of receptor-bound β-D-galactosidase by enzyme assay. Controls adding mannose 6-phosphate with β-D-galactosidase were employed to correct for nonspecific binding of β-D-galactosidase. Additional controls omitting antisera were used to adjust for nonimmunochemical binding of receptors. Crossreaction of either antiserum with nonhomologous purified receptor was 2% of the reaction with homologous receptor.

Purification of α-L-fucosidase

Either B142 or 1141 lymphoid cells (1×10^9) were homogenized by gentle pipeting at 4 °C in 200 ml of 0.15 M sodium acetate, pH 5, containing 0.25% (v/v) Triton X-100. The homogenate was centrifuged at 4 °C at 13 000 × *g* for 0.5 h. The supernatant was collected. The pellet was re-extracted with 50 ml of the buffer, centrifuged again, and the supernatant combined with the previous one. Greater than 95% of α-L-fucosidase activity in crude homogenates was recovered in the combined supernatant fraction and less than 5% was present in the last pellet. Final purification was attained by affinity chromatography on fucosylamine-Sepharose 4B [36].

The biosynthesis and processing of α-L-fucosidase in cultured lymphoid cells is well documented [20–23]. The enzyme is synthesized as a precursor form with a $M_r = 58\,000$ that is processed to either mature intracellular or extracellular forms having $M_r = 60\,000$ and 62 000, respectively. All three forms of α-L-fucosidase are glycoproteins with a common polypeptide chain of $M_r = 52\,000$ but with a different carbohydrate component. The enzyme preparations purified above were estimated to be 80–90% mature, processed intracellular enzyme based upon their molecular masses and the general resistance of the carbohydrate moiety of the enzymes to hydrolysis by endo-H [20–23].

Enzyme and protein assays

α-L-Fucosidase activity was assayed at 37 °C for 0.25 to 2 h. The reaction mixtures contained (in a final volume of 0.1 ml) 0.15 M sodium acetate, pH 5, 0.1% (v/v) Triton X-100, 0.5 mM 4-methylumbelliferyl-α-L-fucoside and either cell (50 to 100 μg) or membrane (20 to 100 μg) protein. The reactions were terminated by addition of 2.9 ml of 0.2 M Na_2CO_3. Fluorescence was measured in a Turner Fluorometer, Model 112, using an excitation filter with $\lambda = 320$–390 nm and an emission filter with $\lambda > 415$ nm. One unit is defined as the release of 1 nmol of substrate per h. Total protein was assayed as described by Bradford [37].

Results

Binding of α-L-fucosidase to intact cells

The binding of α-L-fucosidase to cells was conducted by incubation of catalytically active α-L-fucosidase with cells derived from patients with fucosidosis. The fucosidosis cells were devoid of α-L-fucosidase activity [22, 31, 32, 35], permitting a direct means for the measurement of binding α-L-fucosidase. Recipient cell lines included a fibroblast (JTF) and a lymphoid line (JTL) from the same fucosidosis patient and a lymphoid line (GM) from an unrelated fucosidosis patient. Preliminary experiments demonstrated that binding of α-L-fucosidase to all cells was linear with respect to time (0 to 24 h) and concentration of α-L-fucosidase in medium (71 to 568 units ml^{-1}). Saturable binding of α-L-fucosidase to cells was not attained at the highest concentration of α-L-fucosidase activity tested (568 units ml^{-1}). α-L-Fucosidase activity present in the medium used for binding experiments was stable during 24 h of incubation.

The binding of α-L-fucosidase to fucosidosis fibroblasts (JTF) (122 units per mg cellular protein) was 44-fold greater than binding to lymphoid cells (JTL) derived from the same individual and 10-fold greater than binding to lymphoid cells (GM) from an unrelated patient on a per mg cellular protein basis (Table 1).

Binding of α-L-fucosidase to eiher JTF fibroblasts or GM lymphoid cells was inhibited 70% to 96% by certain phosphorylated saccharides including 1 mM mannose 6-phosphate, L-fucose 1-phosphate, or fructose 1-phosphate, and 0.25 mM phosphomannan (Table 1). However, binding of the enzyme to JTL lymphoid cells was inhibited 5% to

Table 1. Effect of various compounds on binding of lymphoid cell α-L-fucosidase to fibroblasts and lymphoid cells.[a]

Compounds	*Final concentration*	*Cells % Inhibition of binding*[b]		
		JTF	*JTL*	*GM*
Mannose 6-phosphate	(1 mM)	96	5	72
Mannose 6-phosphate	(10 mM)	ND[c]	19	ND
Phosphomannan	(0.25 mM)	95	12	70
Phosphomannan	(2.5 mM)	ND	32	ND
Fucose 1-phosphate	(1 mM)	94	10	83
Fucose 1-phosphate	(10 mM)	ND	11	ND
Fructose 1-phosphate	(1 mM)	93	11	78
Fructose 1-phosphate	(10 mM)	ND	39	ND
Fructose 6-phosphate	(1 mM)	24	2	51
Fructose 6-phosphate	(10 mM)	ND	0	ND
Fructose 1,6-diphosphate	(1 mM)	25	7	31
Fructose 1,6-diphosphate	(10 mM)	ND	16	ND
Glucose 6-phosphate	(1 mM)	16	9	50
Glucose 6-phosphate	(10 mM)	ND	27	ND

[a] Binding measurements were made as described in the Materials and methods section using 1400 units of α-L-fucosidase from B142 lymphoid cells, the indicated compounds, and either fibroblasts (JTF) or lymphoid cells (JTL or GM) from fucosidosis patients. None of the compounds affected the viability of cells.
[b] Binding of α-L-fucosidase from B142 cells to JTF, JTL, and GM cells was 122, 2.8, and 12.6 units per mg cellular protein, respectively. Without normalization versus cellular protein, the percentages of exogenous α-L-fucosidase bound by JTF, JTL, and GM cells were 1.4%, 0.1%, and 0.4%. The following compounds (1 mM final concentration), inhibited binding to each cell line by 10% or less: fructose, mannose, fucose, galactose, sialic acid, *N*-acetylglucosamine, mannose 1-phosphate, glucose 1-phosphate, *N*-acetylglucosamine 1-phosphate, *N*-acetylglucosamine 6-phosphate, galactose 6-phosphate, phosphotyrosine, phosphoserine, and phosphothreonine. Less than 10 % binding inhibition was observed with fucoidan and yeast mannan at 1 mg ml^{-1}.
[c] ND = not determined.

12% by these compounds. Binding of α-L-fucosidase to either JTF fibroblasts or GM lymphoid cells was inhibited 16% to 51% by 1 mM concentrations of other phosphorylated saccharides including fructose 6-phosphate, fructose 1,6-diphosphate, or glucose 6-phosphate, whereas binding to JTL lymphoid cells was inhibited by only 2% to 9% (Table 1). Other naturally occurring and chemically synthesized analogs exhibited little or no inhibitory effect on binding of α-L-fucosidase to any of the three cell lines.

Binding of α-L-fucosidase to cell membranes

The preceding experiments examined the association of α-L-fucosidase with intact cells via the cell surface. However, latent intracellular receptors for α-L-fucosidase might not be detected. Therefore, the binding of α-L-fucosidase to total cell membrane preparations was investigated. Total membrane preparations from cells of nonfucosidosis origin lack endogenous α-L-fucosidase activity. This finding offered a potential means for the detection of novel receptors in ICD and PHP lymphoid cells.

Table 2. Binding of α-L-fucosidase to membranes of lymphoid cells and fibroblasts.[a]

Membrane source	*Binding of α-L-fucosidase*			
	Units bound	*% Inhibition*		
		Man6P[b]	*Glc6P*[b]	*Man1P*[b]
B142	1.1	81	19	15
1141	1.6	89	13	12
1818	2.2	87	11	7
JTL	1.8	80	16	14
GM	1.5	83	15	9
JTF	4.6	88	4	11
WI38	4.4	79	7	9

[a] Binding measurements were made as described in the Materials and methods section using 285 units of α-L-fucosidase derived from B142 cells and 100 μg of membrane protein. B142, 1141, and 1818 are lymphoid cell lines derived from a healthy individual and patients with ICD and PHP, respectively. JTL and GM are lymphoid cell lines derived from fucosidosis patients. JTF is a fucosidosis fibroblast line. WI38 is a normal fibroblast line from normal embryonic lung
[b] 1 mM.

Preliminary experiments with α-L-fucosidase and membranes prepared from B142 cells showed that binding was time dependent from 0 to 1 h and linear with respect to membrane protein (20 to 100 μg per assay). Binding was saturable by 5200 units of α-L-fucosidase to 20 μg of membrane protein. Substitution of 10 mM $MnCl_2$ with 2 mM EDTA in incubation mixtures reduced binding by 30%. Also, the α-L-fucosidase–membrane complex was dissociated by incubation at pH values between 4 and 5 but not at pH values between 6 and 7.

Membranes from lymphoid cell lines JTL (fucosidosis), GM (fucosidosis), 1141 (ICD), 1818 (PHP) and B142 (healthy individual) bound between 1.1 unit and 2.2 units of α-L-fucosidase from B142 cells (Table 2). Membranes from fibroblast lines JTF (fucosidosis) and WI38 (normal embryonic lung) bound 4.6 units and 4.4 units, respectively, of α-L-fucosidase from B142 cells (Table 2). Binding of α-L-fucosidase from B142 cells to each membrane preparation was inhibited greater than 78% by 1 mM mannose 6-phosphate; whereas 1 mM glucose 6-phosphate or 1 mM mannose 1-phosphate was a relatively poor inhibitor of binding (<20%) (Table 2). Furthermore, membranes from B142 lymphoid cells or 1141 (ICD) lymphoid cells failed to bind α-L-fucosidase isolated from 1141 cells (data not shown).

CI-MPR and CD-MPR content of cells

In binding assays conducted with intact cells, the levels of mannose 6-phosphate inhibitable receptor activity at the cell surface were drastically reduced for GM lymphoid cells and virtually absent from JTL lymphoid cells relative to JTF fibroblasts (Table 1). In binding assays with total cell

Table 3. Phosphomannosyl receptor (CI-MPR and CD-MPR) content in human fibroblasts and B-lymphoid cells.[a]

Cell line	*CI-MPR (ng per mg protein)*	*CD-MPR (ng per mg protein)*
Lymphoid		
JTL	0.5	7.2
GM	2.4	15.5
1141	0.8	6.6
1818	2.0	7.8
B142	1.2	6.3
204	0.4	6.4
212	0.8	5.7
219	2.0	16.5
222	0.9	5.1
Mean (SD)	1.2 (0.7)	8.6 (4.3)
Fibroblast		
JTF	21.0	6.9
WI38	25.7	5.4
Mean	23.4	6.2

[a] Measurements were made by the immunoassay procedure described in the Materials and methods section. JTL, GM, and JTF are cell lines derived from fucosidosis patients. Cell lines 1141 and 1818 were derived from ICD and PHP patients, respectively. B142, 204, 212, 219, and 222 were derived from healthy individuals. WI38 was derived from normal embryonic lung.

membranes, substantial mannose 6-phosphate inhibitable receptor activity was found for GM and JTL membranes relative to JTF membranes (Table 2). These results suggest the presence of latent mannose 6-phosphate inhibitable receptors on intracellular membranes of GM and JTL cells. Two distinct phosphomannosyl receptors (CI-MPR and CD-MPR) have been implicated in the intracellular delivery of newly made acid hydrolases to lysosomes but only CI-MPR operates at the cell surface for the binding, internalization, and delivery of exogenous acid hydrolases to lysosomes [2, 3, 30]. Collectively, these observations suggest that the level of CD-MPR is greater than that of CI-MPR in JTL and GM lymphoid cells and that the level of CI-MPR is greater in JTF fibroblasts than in JTL and GM lymphoid cells. Therefore, the occurrence of CI-MPR and CD-MPR in these cells was determined (Table 3). The level of CD-MPR was 14.4-fold greater than that of CI-MPR in JTL cells and 6.5-fold greater than that of CI-MPR in GM cells. The amount of CI-MPR in JTF cells was 42-fold greater than that in JTL cells and 8.8-fold greater than that in GM cells. Thus, the content of CI-MPR and CD-MPR in JTL, GM, and JTF cells correlated with the conclusions inferred from the binding assays that the amount of CD-MPR is greater than that of CI-MPR in JTL and GM lymphoid cells and that the amount of CI-MPR is greater in JTF fibroblasts than in JTL and GM lymphoid cells.

To determine if the increased level of CD-MPR relative to CI-MPR found in JTL and GM cells is a general characteristic of lymphoid cell lines, the level of each receptor was determined in seven additional lymphoid cell lines (Table 3). The mean amount of CD-MPR in nine lymphoid cell lines was 7.2-fold greater than the mean amount of CI-MPR. Thus, the elevated level of CD-MPR relative to CI-MPR is characteristic of lymphoid cell lines.

Discussion

Binding of exogenous α-L-fucosidase to JTF fibroblasts and GM lymphoid cells was specifically inhibited by mannose 6-phosphate and structurally related compounds (Table 1). These results parallel those reported by others for the binding of acid hydrolases to fibroblasts [25–29] and to CI-MPR and CD-MPR [4–9, 38]. Additional evidence suggests that both CI-MPR and CD-MPR bind newly synthesized acid hydrolases intracellularly, but that only CI-MPR binds exogenous acid hydrolases [2, 3, 30]. Together, these results suggest that JTF fibroblasts and GM lymphoid cells bind exogenous α-L-fucosidase via CI-MPR.

A third receptor has been shown to have a binding specificity for mannose 6-phosphate and is involved in the adhesion of lymphocytes to endothelial cells [39]. However, the sulfated polysaccharide fucoidan, derived from brown algae, is a potent binding inhibitor for this receptor [40]. In the present studies, fucoidan failed to inhibit binding of α-L-fucosidase to JTF or GM cells, suggesting that the adhesion receptor was not responsible for binding of α-L-fucosidase to these cells.

The percentage of exogenous α-L-fucosidase derived from B142 cells bound by JTF, GM, and JTL cells was 1.4%, 0.4%, and 0.1%, respectively (Table 1). The percentage bound for JTF fibroblasts was 6-fold to 43-fold lower than binding reported by others using acid hydrolases and fibroblasts [25, 41, 42]. Binding to JTF cells and to GM lymphoid cells was inhibited by mannose 6-phosphate, whereas binding to JTL cells was not. A possible explanation for the low specific binding of α-L-fucosidase to JTF and GM cells is that only a small portion of the α-L-fucosidase molecules obtained from B142 cells carry the mannose 6-phosphate recognition marker. This hypothesis is supported by our studies, which indicate that the carbohydrate portion of the majority of mature, intracellular α-L-fucosidase from B142 cells is resistant to hydrolysis by endo-H [22, 23]. It has been reported that mannose 6-phosphate residues are only found on carbohydrate chains sensitive to hydrolysis by endo-H [2, 3, 24]. In addition, less than 1% of $^{32}P_i$ incorporated into newly synthesized α-L-fucosidase of B142 cells is present as mannose 6-phosphate residues [21]. A possible explanation for the increase in specific binding of α-L-fucosidase to JTF cells as compared to GM and JTL cells is that the level of CI-MPR is 8.8-fold greater in JTF cells than that in GM cells and 42-fold greater in JTF cells than that in JTF cells (Table 3). Additionally, the low (and apparently nonspecific)

binding of α-L-fucosidase obtained from B142 cells to JTL lymphoid cells suggests that the association of α-L-fucosidase with JTL cells is not receptor mediated and may be the result of fluid phase endocytosis [28, 43].

The binding of α-L-fucosidase from B142 cells to membranes prepared from lymphoid cells of a healthy individual (B142) and a patient with ICD (1141) was specifically inhibited by mannose 6-phosphate (Table 2), suggesting the presence of phosphomannosyl receptors on the membrane preparations from each cell line. Membranes from B142 cells and 1141 cells failed to bind α-L-fucosidase derived from 1141 cells, suggesting that α-L-fucosidase from 1141 cells lacks mannose 6-phosphate. This is consistent with the observed absence of mannose 6-phosphate residues on α-L-fucosidase derived from 1141 cells [21] and the observed deficiency of *N*-acetylglucosamine 1-phosphotransferase activity in this cell line [18].

Despite the lack of mannose 6-phosphate on the α-L-fucosidase of 1141 cells, these cells maintain normal intracellular levels of α-L-fucosidase [18, 20, 21]. This result strongly suggests that a phosphomannosyl independent mechanism for intracellular retention of α-L-fucosidase must exist in this cell line. However, attempts to identify receptors in lymphoid cells capable of binding α-L-fucosidase independently of mannose 6-phosphate were unsuccessful.

CI-MPR and CD-MPR have been implicated in the intracellular trafficking of newly made acid hydrolases [2, 3]. The mean level of CD-MPR was 7.2-fold greater than mean level of CI-MPR in nine human B-lymphoid cell lines (Table 3). The mean level of CI-MPR was 3.8-fold greater than CD-MPR in two fibroblast lines. Furthermore, the mean level of CI-MPR was 19.5-fold greater in the fibroblast lines than in the lymphoid cell lines assayed; whereas the level of CD-MPR determined in the fibroblast and lymphoid cell lines was comparable. Six previously examined murine B-lymphoid cell lines were deficient in CI-MPR activity relative to CI-MPR activity in human fibroblasts [11], while human fibroblasts synthesize 4- to 10-fold more CI-MPR than CD-MPR [12]. These results strongly suggest that B-lymphoid cells are deficient in CI-MPR and that regulation of expression of CI-MPR and CD-MPR in B-lymphoid cells and fibroblasts differ. The difference is also noted in the JTL and JTF cell lines which have the same genomes.

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