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Glycosyltransferase Activities in Leukemic Cells from Patients and Human Leukemic Cell Lines*

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Abstract—*The cell membrane fraction from c-ALL, B-ALL, Ph'+ ALL, B-CLL, T-CLL, AML, blastic-CML, normal leukocytes, PHA-stimulated lymphocytes and several T, B and myeloid human leukemic cell lines has been used in different cell types to demonstrate different patterns of glycosyltransferase activity. Both B- and T-CLL cell membranes have low fucosyltransferase B and A activity compared to acute leukemias; while sialyltransferase activity is higher in B- than in T-CLL. AML cell membranes and ML-1 human myeloblast cell line membranes have exceptionally high fucosyltransferase A activity compared to all other leukemic cells or cell lines. Human leukemic B cell lines expressed cell membrane sialyltransferase, fucosyltransferase B and probably fucosyltransferase A activity several times higher than T cell lines. Human myeloid cell lines ML-1 and HL-60 express 5- to 20-fold higher galactosyltransferase activity than human leukemic T and B cell lines. Both sialyltransferase and galactosyltransferase activity were higher in all leukemic cells than in normal leukocytes and PHA-stimulated normal lymphocytes. This is the first study carried out on glycosyltransferases using cells obtained from leukemic patients characterized immunologically. These results indicate that all glycosyltransferase activity, with the exception of fucosyltransferase activity in CLL, were higher in leukemic cells than in normal cells. Moreover, large differences in these enzymes, e.g. very high galactosyltransferase activity in myeloid cell lines compared to B and T cell lines, of fucosyltransferase A in AML and myeloblast cell lines compared to all other cells, and of sialyltransferase in B-CLL or B cell lines compared to T-CLL or T cell lines, could be useful in characterizing certain leukemias and hematopoietic cell lines.*

INTRODUCTION

GLYCOSYLTRANSFERASES are primarily membrane-bound enzymes that are located in the endoplasmic reticulum, Golgi system [1, 2], and plasma membrane [3, 4] of most cells. Because of their specific function in the biosynthesis of membrane glycoconjugates, the glycosyltransferases are considered to be intimately involved with neoplastic cell growth. Elevated sialyltransferase [5, 6] galactosyltransferase [7] and fucosyltransferase [8] activity has been found in the plasma from tumor-bearing animals. In addition, the levels of plasma sialyltransferase and GDP-fucose galactoside fucosyltransferase have been found to be increased in myelopoietic and lymphosarcomatous diseases [9–11]. Greater sialyltransferase activity has been found in CLL cells than in normal leukocytes [12, 13]. Although the plasma fucosyltransferase B activity in normal subjects and those with untreated or unresponding AML or lymphoma was comparable, it was increased in AML or lymphoma patients during drug-maintained remissions [10]. This enzyme

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Abbreviations: AML = acute myeloblastic leukemia; APL = acute promyelocytic leukemia; c-ALL = common form of acute lymphoblastic leukemia; Ph'+ ALL = Philadelphia chromosome-positive acute lymphoblastic leukemia; CLL = chronic lymphocytic leukemia; CML-BP = blastic phase of chronic myelocytic leukemia; BL = Burkitt's lymphoma; PHA = phytohemagglutinin; B- = bone marrow-derived; T- = thymus-derived; HEPES = *N*-2-hydroxyethylpiperazine-*N'*-2-ethanelsulfonic acid; fucosyltransferase A = enzyme catalyzing the transfer of fucose onto galactose residue of asialofetuin acceptor; fucosyltransferase B = enzyme catalyzing the transfer of fucose onto *N*-acetylglucosamine (asialo agalactofetuin).

was not detected in CLL cell extracts [14]. Since most of these studies were carried out with plasma, uncertainties about the origin of these enzymes have existed. In this study we have examined sialyltransferase, galactosyltransferase and fucosyltransferase A and B activity in the cell membrane fraction isolated from various normal and leukemic human cells and cell lines to determine their association with particular cell types. Our results indicate that cell membranes of the various hematopoietic cells examined display a specific pattern of glycosyltransferases that could be useful for their characterization.

MATERIALS AND METHODS

Clinical materials and cell lines

Leukocytes were obtained by leukapheresis from three ALL patients (B-ALL, c-ALL, Ph'+ ALL), three CLL patients (two B-CLL and one T-CLL), one AML patient and one with myeloid-type blastic phase CML [15]. Blastic phase CML and all acute leukemia patients had 90–99% blasts in the peripheral blood, whereas all three CLL patients had 85–90% lymphocytes in the peripheral blood. The white blood cell count for all leukemic patients was between 71,000 and 196,000/μl and leukapheresis was used to reduce the leukemic cell burden. The c-ALL, Ph'+ ALL and blastic CML patients were high in terminal transferase activity, whereas all others were negative for terminal transferase. The immunological phenotyping of leukemic cells was carried out as described by Minowada *et al.* [16]. After the removal of erythrocytes by dextran sedimentation, the leukocytes were recovered by centrifugation.

Normal leukocytes (*ca.* 65% granulocytes, 30% lymphocytes and 5% monocytes), lymphocytes, monocytes and granulocytes were obtained from the buffy coats of healthy donors by standard techniques, including dextran sedimentation, Ficoll-Hypaque gradient centrifugation and adherence of monocytes to glass. To obtain stimulated lymphocytes, the normal lymphocytes (2×10^6 cells/ml in RPMI 1640 medium containing 5% heat-inactivated fetal calf serum, penicillin 100 units/ml and streptomycin 50 μg/ml) were incubated at 37°C for 72 hr with PHA-M (Difco Laboratories, Detroit, MI) at a final concentration of 1/50.

Three T-ALL cell lines (Molt-4, CCRF-HSB-2, CEM-C_1), one Burkitt's lymphoma B cell line (HR1K), three myeloid cell lines (ML-1, HL-60 and K-562, representing myeloblastic, promyelocytic and pre-erythroblastic cells respectively), two B cell lines of ALL origin (RPMI-8392; RPMI-8432) and one B cell line of normal origin (RPMI-1788) were used. The characteristics of these cell lines have been described [16, 17]. All cell lines were grown in RPMI 1640 medium containing 5% heat-inactivated fetal calf serum and maintained in the log phase of growth by appropriate feeding. All cells were washed several times with phosphate-buffered saline and stored at -80°C until used.

Total cell membrane preparation

For total cellular membrane preparation, about 2 g of cells were suspended in 0.25 M sucrose, buffered with Tris–HCl (0.05 m), pH 7.2, and homogenized manually in a glass homogenizer until more than 85% of cells were broken. The homogenate was filtered through cheesecloth and overlayed on 2.0 M sucrose, buffered with 0.05 M Tris–HCl, pH 7.2. Gradients were centrifuged at 24 krev/min for 2 hr in a Beckman Model L ultracentrifuge.

The cellular membrane fraction, which banded at the interphase, was collected and suspended in 0.05 M HEPES, pH 6.5. To remove sucrose, membranes were washed with 0.05 M HEPES, pH 6.5, and sedimented by centrifugation for 30 min at 24 krev/min. Before use, membranes were sonicated for 15 sec using a Biosonic i.v. sonicator and diluted to the protein concentration of about 100 μg/10 μl. Protein concentration was estimated using the Bio-Rad protein assay as described by Bradford [18]. Based on adenosine 5'-α,β methylene diphosphonate-sensitive 5'-nucleotidase activity determinations (a plasma membrane marker enzyme [19]), the recovery of the membrane fraction from the cells was 60–70%.

Glycosyltransferase assays

Glycosyltransferase activity was determined according to the following established procedures [11, 12, 20]: sialyltransferase activity was measured in the medium containing 0.2 mg of asialofetuin, 0.05 M HEPES, pH 6.5, 0.1 μCi CMP-sialic acid (sialic-4,5,6,7,8,9-[^{14}C] sp. act. 213 mCi/mmol, New England Nuclear), 10 mM $MgCl_2$, 0.1% Triton X-100 and 10 μl of cell membrane suspension. The total volume was adjusted to 100 μl with distilled water. Incubation was continued for 60 min at 37°C. Incubation was terminated by the addition of 1 ml of 1% phosphotungstic acid in 0.5 M HCl. Precipitates were washed three times with 10% trichloroacetic acid and finally once with absolute methanol. The pellets were solubilized in 0.5 ml of NCS solution (Amersham), mixed with 10 ml of toluene-based scintillation fluid and counted using a Packard counter.

Galactosyltransferase activity was assayed in the medium containing: 0.1 M cacodylate buffer, pH 7.4, with 0.15 M NaCl, 10 mM $MnCl_2$, 0.2 mg of asialoagalactofetuin, 0.1 μCi UDP-galactose

(galactose-[^{14}C-U], sp. act. 302 mCi/mmol, New England nuclear) and 10 μl of cell membranes suspension.

Samples were incubated for 60 min at 37°C and processed as above.

Fucosyltransferases activity was tested in medium composed of: 0.05 M cacodylate buffer, pH 7.0, 1 mM neutralized ATP-disodium salt (Sigma Chemical Co.), 10 mM $MgCl_2$, 0.1 μCi GDP-fucose (fucose [^{14}C-U], sp. act. 192 mCi/mmol, New England Nuclear), 10 μl of cell membranes suspension and 0.2 mg of asialofetuin (fucosyltransferase A) or 0.2 mg of asialoagalactofetuin (fucosyltransferase B).

Samples were incubated for 60 min at 37°C and processed as before (see sialyltransferase assay). In all samples endogenous glycosyltansferase activity was assayed in the appropriate incubation medium devoid of exogenous substrate. All samples were studied in triplicate using duplicate tests for each sample.

Preparation of exogenous acceptors

Fetuin was obtained from Sigma Chemical Co., St. Louis, MI. Terminal sialic acid and penultimate galactose residues were removed from fetuin by the method described by Kim *et al.* [21] and as modified by Podolsky and Weiser [20].

Fetuin was suspended in 0.1 M H_2SO_4 and incubated at 80°C for 50 min. The hydrolysate was neutralized by the addition of 1 N NaOH and then dialysed against water at 2°C. The sialic acid-free fetuin was then suspended in 0.01 M sodium metaperiodate in 0.05 M sodium acetate buffer, pH 4.5, and incubated for 24 hr in the dark at 2°C. The reaction was stopped by the addition of glycerol and the suspension was then dialysed against water. The non-diffusible material was freeze-dried and then suspended in 0.15 M $NaBH_4$ and 0.15 M potassium tetraborate, pH 8.0, for 14 hr at 4°C. The reaction was stopped by adjusting the pH to 5.0 by addition of 1 M acetic acid and the material was then dialysed against water. The non-diffusible, galactose-free fetuin was then freeze-dried.

RESULTS

Activity of sialyltransferase

Endogenous sialyltransferase activity in cell membrane fraction was low from leukemic cells, from normal human leukocytes and from PHA-stimulated lymphocytes (Table 1). However, both endogenous sialyltransferase activity and the activity measured in the presence of exogenous acceptor (desialylated fetuin) was higher in ALL, AML and B-CLL than in T-CLL, normal leukocytes or PHA-stimulated normal lymphocytes.

The sialyltransferase activity in T-CLL was comparable to that in normal leukocytes and PHA-stimulated normal lymphocytes.

Among human cell lines, a 2- to 3-fold higher sialyltransferase activity was found in B cell lines than in the T cell line (Table 2). However, the highest sialyltransferase activity with exogenous substrate was found in promyelocytic leukemia cell line HL-60 and in the B-cell line of normal origin (RPMI-1788).

Activity of galactosyltransferase

The distribution pattern of galactosyltransferase activity among leukemic and normal blood leukocytes was similar to that of sialyltransferase. All ALL, B-CLL and AML cell membrane fractions exhibited galactosyltransferase activity several-fold higher than normal leukocytes and PHA stimulated lymphocytes (Table 3). T-CLL cell membranes were the only exception, exhibiting galactosyltransferase activity more similar to normal cells.

Although there were no consistent differences in endogenous galactosyltransferase activity

Table 1. Activity of sialyltransferase in cell membrane fractions from leukemic cells from patients

Cells	No acceptor	Desialylated fetuin acceptor	Difference
	(pmol [^{14}C]-sialic acid incorporated/mg protein/hr)		
Ph'+ ALL	13.7 ± 1.8	507.3 ± 31.9	493.6
C-ALL	11.5 ± 1.9	201.2 ± 4.2	189.7
B-ALL	18.8 ± 2.3	462.7 ± 29.5	443.9
B-CLL (I)	7.8 ± 0.3	381.7 ± 22.6	373.9
B-CLL (II)	7.1 ± 2.2	95.0 ± 8.9	87.9
T-CLL	2.3 ± 0.4	28.8 ± 3.2	26.5
AML	7.3 ± 2.4	91.0 ± 14.1	83.7
Normal leukocytes	3.2 ± 0.6	21.1 ± 0.7	19.9
PHA-stimulated normal lymphocytes	4.8 ± 0.9	24.8 ± 0.8	20.0

The values in this and all other tables represent mean ± standard deviation for three separate determinations.

Table 2. Activity of sialyltransferase in cell membrane fractions from human hematopoietic cell lines

Cell lines	Origin	Cell type	No acceptor (pmol [^{14}C]-sialic acid incorporated/mg protein/hr)	Desialylated fetuin acceptor (pmol [^{14}C]-sialic acid incorporated/mg protein/hr)	Difference (pmol [^{14}C]-sialic acid incorporated/mg protein/hr)
Molt-4	ALL	T	3.3 ± 0.3	18.2 ± 1.2	14.9
CCRF-HSB$_2$	ALL	T	5.5 ± 0.6	26.6 ± 4.2	21.1
CEM-C$_1$	ALL	T	2.8 ± 0.4	14.3 ± 1.9	11.5
RPMI-8392	ALL	B	5.8 ± 0.1	44.8 ± 1.6	39.0
RPMI-8432	ALL	B	6.6 ± 1.0	43.2 ± 6.9	36.6
HR1K	BL	B	5.1 ± 0.9	52.4 ± 4.3	47.3
RPMI-1788	Normal	B	7.9 ± 0.8	192.1 ± 20.0	184.2
HL-60	APL	promyelocyte	3.3 ± 0.5	348.9 ± 13.5	345.6

Cell lines RPMI-8392 and RPMI-8432, although of ALL origin, are not considered to represent leukemic cells.

Table 3. Activity of galactosylotransferase in cell membrane fractions from leukemic cells from patients

Cells	No acceptor (pmol [^{14}C]-galactose incorporated/mg protein/hr)	Sialic acid- and galactose-free fetuin acceptor (pmol [^{14}C]-galactose incorporated/mg protein/hr)	Difference (pmol [^{14}C]-galactose incorporated/mg protein/hr)
PH'+ ALL	31.3 ± 5.0	193.7 ± 23.1	162.4
C-ALL	28.1 ± 4.8	101.4 ± 5.4	73.3
B-ALL	95.8 ± 15.3	479.3 ± 42.1	383.5
B-CLL (I)	42.2 ± 3.5	276.7 ± 9.5	234.5
B-CLL (II)	30.2 ± 1.5	164.2 ± 8.1	134.0
T-CLL	13.8 ± 1.1	43.9 ± 1.9	30.1
AML	39.3 ± 2.9	102.7 ± 15.1	68.4
Normal leukocytes	6.5 ± 0.5	26.1 ± 4.0	19.6
PHA-stimulated normal lymphocytes	12.1 ± 1.0	24.7 ± 3.4	12.6

between T, B and myeloid human leukemic cell lines, the activity with exogenous substrate was about 5- to 20-fold higher in the two myeloid cell lines ML-1 and HL-60 than in the other cell lines (Table 4). In addition, the activity in the B cell line of normal origin (RPMI-1788) was 2–4 times higher than in the B cell lines of ALL or Burkitt's lymphoma origin.

Activity of fucosyltransferases

Fucosyltransferase A and B activity differs significantly between ALL and CLL cell membranes (Table 5). Fucosyltransferase A activity in the ALL cell membrane fraction was 5-fold higher than CLL cell membranes on average. Also, it was found that fucosyltransferase B activity was very low or undetectable in the CLL cell membranes, while activity of ALL cell membranes was about one-half of fucosyltransferase A and only slightly higher than in normal leukocytes and PHA-stimulated normal lymphocytes (Table 5). Although AML and CML blastic cells had high activity of fucosyltransferase B like the ALL cells, the activity of fucosyltransferase A in AML cells was very high compared to all the other cells. On the other hand, fucosyltransferase B with exogenous acceptor was not detectable in PHA-stimulated normal lymphocytes.

Cultured human B cell lines exhibit activity of both fucosyltransferases and endogenous activity that is several-fold higher than the T cell lines (Table 6). The highest fucosyltransferase A activity was found in the ML-1 myeloblast cell line, which was more than 3-fold higher than in the more mature promyelocytic cell line HL-60, and was very close to the value of fucosyltransferase A activity found in the cell membrane fraction from AML patients (Table 5).

DISCUSSION

Leukemic cells from ALL, CLL, AML and blastic phase CML display different levels of cell membrane glycosyltransferase activity. Estimation of sialyltransferase activity in membrane fractions of clinical samples shows high enzyme activity in all ALL, AML and B-CLL samples. The T-CLL leukemic cell membrane fraction showed the lowest activity of sialyltransferase, which was similar to the normal leukocytes and PHA-stimulated lymphocytes. Steimer and Despont [13] presented results indicating that

Table 4. Activity of galactosylotransferase in cell membrane fractions from human hematopoietic cell lines

Cell lines	Origin	Cell type	No acceptor (pmol [^{14}C]-galactose incorporated/mg protein/hr)	Sialic acid- and galactose-free fetuin acceptor (pmol [^{14}C]-galactose incorporated/mg protein/hr)	Difference (pmol [^{14}C]-galactose incorporated/mg protein/hr)
Molt-4	ALL	T	57.3	92.2 ± 4.9	34.9
CCRF-HSB$_2$	ALL	T	27.9	52.8 ± 2.0	24.9
RPMI-8392	ALL	B	32.0 ± 3.2	53.5 ± 2.1	21.5
RPMI-8432	ALL	B	57.2 ± 2.3	69.0 ± 8.6	11.8
HR1K	BL	B	25.4 ± 2.6	36.1 ± 0.5	10.7
RPMI-1788	Normal	B	36.9 ± 2.0	85.9 ± 13.1	49.0
ML-1	AML	myelobast	12.5 ± 2.9	237.1 ± 30.1	224.6
HL-60	APL	promyelocyte	44.0 ± 8.9	278.7 ± 22.5	234.7

Table 5. Activity of fucosyltransferases A and B in cell membrane fractions from human cells from patients

Cells	No acceptor	Sialic acid-free fetuin acceptor (A)	Difference	Sialic acid- and galactose-free fetuin acceptor (B)	Difference
	(pmol of [^{14}C]-fucose incorporated/mg protein/hr)				
Ph'+ ALL	26.2 ± 1.7	58.8 ± 3.4	32.6	38.6 ± 11.2	12.4
C-ALL	8.7 ± 0.8	54.2 ± 2.2	45.5	21.8 ± 0.8	13.1
B-ALL	7.0 ± 1.0	20.0 ± 0.5	13.0	24.0 ± 1.3	17.0
B-CLL (I)	3.1 ± 1.0	9.3 ± 0.5	6.2	7.0 ± 2.1	3.9
B-CLL (II)	3.5 ± 0.3	10.7 ± 5.2	7.2	3.0 ± 0.8	0
T-CLL	3.1 ± 0.1	7.6 ± 0.2	4.5	4.2 ± 2.0	1.1
AML	20.0 ± 8.2	115.0 ± 7.0	95.0	35.0 ± 1.0	15.0
CML blastic cells	7.3 ± 2.3	25. 0 ± 2.7	17.7	14.0 ± 3.0	6.7
Normal leukocytes	2.8 ± 0.6	10.2 ± 0.6	7.4	11.0	8.2
PHA-stimulated normal lymphocytes	21.6 ± 2.9	20.4 ± 0.2	0	31.6	10.0
Monocytes	3.7	7.6	3.9	4.5 ± 0.7	0.8
Granulocytes	2.6 ± 0.3	21.5	18.9	6.3	3.7

Table 6. Activity of fucosyltransferases A and B in cell membrane fractions from human hematopoietic cell lines

Cell lines	Origin	Cell type	No acceptor	Sialic acid-free fetuin acceptor (A)	Difference	Sialic acid- and galactose-free fetuin acceptor (B)	Difference
			(pmol [^{14}C]-fucose incorporated/mg protein/hr)				
Molt-4	ALL	T	5.3 ± 0.6	12.9 ± 2.1	7.6	9.5 ± 3.1	4.2
CCRF-HSB$_2$	ALL	T	4.2 ± 0.5	9.8 ± 0.8	5.6	7.0 ± 0.2	2.8
RPMI-8392	ALL	B	14.9 ± 3.5	29.4 ± 0.5	14.5	29.6 ± 2.5	14.7
RPMI-8432	ALL	B	22.0 ± 3.0	28.4 ± 2.1	6.4	39.6 ± 4.2	17.6
RPMI-1788	Normal	B	34.6 ± 0.5	44.6 ± 2.9	10.0	49.3 ± 1.3	14.7
ML-1	AML	myeloblast	46.3 ± 13.5	144.5 ± 15.6	98.2	52.9 ± 15.8	6.6
HL-60	APL	promyelocyte	6.9 ± 0.2	36.0 ± 2.2	29.1	23.2 ± 1.8	16.3
K562	CML-BP	pre-erythroblast	4.0 ± 0.9	18.2 ± 2.3	14.2	6.9 ± 0.9	2.9

sialyltransferase activity in CLL (B cell?) lymphocytes was several times higher than normal T and B lymphocytes and blood monocytes. Kessel *et al.* [12], using a similar assay system as that reported here, found high sialyltransferase activity in CLL (B cell?) cell extracts. Our results with B-CLL support these findings.

Elevations in serum sialyltransferase activity have been found in many human malignant diseases [6, 12, 22, 23] and in tumor-bearing animals [5, 24]. Although serum sialyltransferase may be shed or secreted into the circulatory system, it is difficult to show direct comparison, since we have used isolated cell membrane fractions. Nevertheless, all leukemia samples in our study had sialyltransferase activity significantly higher than normal leukocytes or PHA-

stimulated normal lymphocytes. Among cell lines, the cell membrane fraction from B cell lines had several-fold higher sialyltransferase activity than T cell lines. This is in agreement with the higher ectosialyltransferase activity in two human B cell lines than in the two T cell lines reported earlier [25]. That T cells may have lower sialyltransferase activity than B cells is also illustrated by lower sialyltransferase activity in T-CLL than in B-CLL (Table 1). Further confirmation of this finding with T-CLL is not possible since we had no additional T-CLL patients at this institute in the last several years.

Galactosyltransferase activity was significantly higher in the cell membrane fraction from all leukemic cells than in normal leukocytes or PHA-stimulated normal lymphocytes, although T-CLL had lower activity than B-CLL. These results are in agreement with elevations in galactosyltransferase activity found in cancer cells [26, 27]. Among the cell lines, all of the myeloid lines had very high galactosyltransferase activity compared to all other cell lines that could be useful in their characterization.

The most significant differences between ALL and CLL cells were in fucosyltransferase B activity. The mean level of fucosyltransferase A was about 6-fold lower in CLL than in ALL samples. Fucosyltransferase B activity was the lowest in the CLL membrane fraction, in contrast to the ALL fraction. The lack of fucosyltransferase B activity in lymphocytic extracts from CLL patients was reported before by Chou and Kessel [14].

Results presented here indicate that fucosyltransferase activity, especially fucosyltransferase B, could be useful in differentiating acute and chronic lymphocytic leukemias. On the other hand, fucosyltransferase A activity was found to be several times higher in the AML cell membrane fraction than in the ALL cell membrane fraction, and could be useful in distinguishing ALL from AML. Elevated levels of plasma fucosyltransferase A have been reported [11] for patients with AML. Like AML cells from a patient, the myeloid cell line ML-1, consisting of myeloblasts, had several times higher fucosyltransferase A activity than all other cell lines. Moreover, the more mature promyelocytic cell line HL-60 and granulocytes had lower activity than the myeloblast cell line ML-1, indicating that this enzyme may decline with maturation along the granulocyte series.

The results presented in this paper represent the first study on glycosyltransferases with cells from leukemic patients characterized immunologically and their comparison with leukemic cell lines. The results indiate that various leukemic cells from patients and leukemic cell lines show a distinct pattern of cell membrane glycosyltransferase activity, some of which could be useful for their characterization. However, further work with a larger patient population would be required to develop some observed differences as markers for the characterization of certain leukemias.

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