Br. J. Cancer (1983), **47**, 158–160

Short Communication

Ectosialyltransferase Activity: A Marker for certain human haematopoietic cells

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Ectosialyltransferase which can add sialic acid to cell surface acceptor proteins is found in many cells and its activity is elevated in transplantable and metastasizing tumour cells (Dobrossy *et al.*, 1981). Its presence on the surface of L1210 leukaemia cells has been confirmed ultrastructurally (Porter & Bernacki, 1975). Recently Maca & Hakes (1978) have reported high activity of this enzyme in two human B-cell lines as compared to two human leukaemic T-cell lines. In order to determine the value of this enzyme as a marker for certain haematopoietic cells or their differentiation, we have examined ectosialyltransferase activity in 23 immunologically characterized human cell lines of normal and various leukaemia/lymphoma origin which represent cells at different levels of maturation (Minowada *et al.*, 1981).

Twenty-three human cell lines, 3 of normal and 20 of various leukaemia/lymphoma origin were examined. The immunological characteristics of these cell lines, their origin and their differentiation stage have been described (Minowada *et al.*, 1981). All cell lines were grown in RPMI 1640 medium containing 5% heat inactivated foetal calf serum and maintained in log phase of growth by appropriate feeding. At harvest, the cell viability as determined by trypan blue exclusion test was 90–95%. Ectosialyltransferase activity was determined according to the procedure of Maca & Hakes (1978) with some modifications. Pelleted cells were washed with 50 mm N-2-hydroxy ethyl piperazine-N', 2-ethanesulfonic acid (HEPES), pH 6.5 containing 0.9% NaCl and 10 mm $CaCl_2$. One half of the cells remained untreated whereas the other half of cells was treated with 10 units of *Vibrio cholerae* neuraminidase (Calbiochem. Behring Corp.) for 30 min at 37°C, pelleted by centrifugation (800 g) and washed with 50 mm HEPES, pH 6.5, containing 0.9% NaCl. Untreated and neuraminidase treated cells (5×10^6) were suspended in 0.5 ml of 50 mM HEPES, pH 6.5–0.9% NaCl containing 0.1 μCi of CMP-sialic acid [sialic—4, 5, 6, 7, 8, 9-^{14}C, specific activity 213 mCi mm^{-1}, New England Nuclear] and incubated for 60 min at 37°C. The reaction was terminated by addition of 2 ml of 1% phosphotungstic acid in 0.5 N HCl and centrifugation. The precipitated material was washed 3 × with 5% trichloroacetic acid followed by 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.

All human cell lines examined here (Table) contained endogenous ectosialyltransferase activity as measured by incorporation of N-(acetyl-^{14}C) neuraminic acid from CMP-N-(acetyl-^{14}C) neuraminic acid to cell surface acceptor proteins. Moreover, as reported for mouse cell line L1210 (Bernacki, 1974) and human cell line Raji (Kilton & Maca, 1977), neuraminidase treatment of cells prior to labelling increased assembly of cell surface sialoproteins several times in most of the cell lines. Both endogenous ectosialyltransferase activity and that obtained after neuraminidase treatment were 2–4 times higher in B-cell lines including plasma cell line RPMI-8226 as compared to T-cell lines which is in agreement with the finding of Maca & Hakes (1978) with two T-acute lymphoblastic leukaemia (T-ALL) and two B-cell lines. No differences in above enzyme activity were found between B-cell lines of normal and malignant origin or between T-ALL cell lines representing T-blasts and T-chronic lymphocytic leukaemia (T-CLL) line SKW-3 representing more mature cells. On the other hand, pre-B cell lines NALM-1 and NALM-6 as well as non-T/non-B cell line NALM-16 expressed lower ectosialyltransferase activity, as compared to B-cell lines. In addition to B-cell lines the pre-erythroblast cell line K562 (Lozzio & Lozzio, 1979) and pre-myeloblast cell line KG-1 (Koeffler *et al.*, 1981) also had higher ectosialyltransferase activity whereas more mature myeloid/monocytoid lines ML-2, ML-3, HL-60, and U-937 had activity comparable to T-cell lines. These results indicate that ectosialyltransferase activity is lower in human T-cell lines compared to

Received 21 July 1982; accepted 28 September 1982.

0007-0920/83/010158-03 $01.00

Table Ectosialyltransferase activity of human haematopoietic cell lines

		CPM 10^{-7} *Cells*		
Cell Line	*Origin*	*Untreated*	*Neuraminidase Treated Cells*	*Treated Cells Minus Untreated*
T-Cell Lines:				
CCRF-CEM	ALL	154±40	290±6	136
CEM-A8	ALL	102±17	198	96
RPMI-8402	ALL	122±15	329±30	207
CCRF-HSB_2	ALL	174±25	274±118	100
MOLT-4	ALL	152	377±112	225
SKW-3	CLL	117±33	189±35	72
B-Cell Lines:				
RPMI-8057	Normal	202±24	892±16	630
RPMI-1788	Normal	309±48	624±93	315
B-89	Normal	144±18	664±75	520
RPMI-8392	ALL	256±18	604±56	348
HRIK	BL	258±6	466±46	208
U698M	LS	370±33	1284±187	914
JOK-1	HCL	378±6	1140±217	762
RPMI-8226	MM	697±66	1458±213	761
Pre-B-Cell Lines:				
NALM-1	CML-BP	134±21	192±38	58
NALM-6	ALL	126±16	346±78	220
Non-T-Non B-Cell Line:				
NALM-16	ALL	170±14	426±18	256
Myeloid Cell Lines:				
K-562	CML-BP	204±26	1572±777	1368
KG-1	AML	570	1036±180	466
ML-2	AML	148±20	338±130	190
ML-3	AML	165±12	253±16	88
HL-60	APL	135±24	352±28	217
U-937	HL (Monoblastoid)	177±8	346±105	169

The above values represent mean ± standard deviation for 3 separate determinations. Values without standard deviation are means of 2 determinations. T-cell lines form non-immune rosettes with sheep red blood cells, B-cell lines have cell surface immunoglobulin, pre-B-lines have cytoplasmic immunoglobulin M and non-T/non-B line lacks T-, B-, or myeloid cell markers (Minowada *et al.*, 1981). ALL = acute lymphocytic leukaemia; CLL = chronic lymphocytic leukaemia; AML = acute myelocytic leukaemia; APL = acute promyelocytic leukaemia; CML-BP = blastic phase of chronic myelocytic leukaemia; BL = Burkitt's lymphoma; LS = lymphosarcoma; HCL = hairy cell leukaemia; MM = multiple myeloma; HL = histiocytic lymphoma.

B-cell lines and that this activity may increase along B-cell series and decrease along myeloid series with maturation. The above results most probably reflect different patterns of cell surface glyconjugates which have been proposed to be characteristic for different hematopoietic cells (Nilsson *et al.*, 1977; Krusius *et al.*, 1979; Klock *et al.*, 1981).

Supported by USPHS Grants CA-17140 and CA13038. The authors wish to thank Dr. J. Minowada for the supply of cell lines used in this study.

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