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 CaCl₂. 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 x 10⁶) 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, 9-14C, 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 x 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 ectosialyltransfase activity as measured by incorporation of N-(acetyl-14C) neuraminic acid from CMP-N-(acetyl-14C) 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 (Koeffer 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...
Table  Ectosialyltransferase activity of human haematopoietic cell lines

| Cell Line          | Origin | Untreated | Neuraminidase Treated Cells | Treated Cells Minus Untreated |
|--------------------|--------|-----------|----------------------------|-----------------------------|
| 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                         |
| 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-HSB₂          | 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-8507          | 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     | 177 ± 8   | 346 ± 105                  | 169                         |
| (Monoblastoid)     |        |           |                            |                             |

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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