Vol. 86, No. 4, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1979 Pages 1281-1287

CELL SURFACE CHARGE ALTERATIONS OCCURRING DURING DIMETHYLSULFOXIDE-INDUCED ERYTHRODIFFERENTIATION OF FRIEND LEUKEMIA CELLS[1]

Alfred E. Brown,[2] Kenneth R. Case, H. Bruce Bosmann[3] and Alan C. Sartorelli

Department of Pharmacology and Developmental Therapeutics Program, Comprehensive Cancer Center, Yale University School of Medicine, New Haven, CT 06510 (AEB, ACS) and Department of Pharmacology and Toxicology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14620 (KRC, HBB)

Received January 24, 1979

SUMMARY:

Dimethylsulfoxide-induced erythrodifferentiation of Friend leukemia cells caused a decrease in net negative cell surface charge which began two days after exposure to the polar solvent and continued throughout the maturation process. Neuraminidase treatment caused a marked reduction in mobility of both untreated and dimethylsulfoxide-treated cells suggesting that sialic acid residues are the major anionogenic moieties of the surface membrane of Friend cells. A decrease in the content of total glycosidically bound sialic acid in dimethylsulfoxide-treated cells also occurred. The findings provide evidence to support an association between erythrodifferentiation of Friend cells and net negative surface charge dependent upon sialic acid residues.

INTRODUCTION:

Friend leukemia cells (FLC) are neoplastic cells that can be induced to differentiate in vitro along the erythroid pathway by treatment with dimethylsulfoxide (DMSO)[4] or other chemical agents (1). The induced FLC are characterized by a reduction in proliferative capacity (1-3), and morphological and biochemical changes similar to those associated with the differentiation of proerythroblasts to orthochromatic normoblasts (4). These findings have included a number of alterations in external surface membranes, such as the appearance and disappearance of certain mouse erythrocyte antigens (5,6), changes in membrane permeability (7,8), changes in agglutinability by various lectins (9,10), and an increase in the synthesis of spectrin (11).

[1] This research was supported in part by U.S. Public Health Service Grants CA-05349, CA-02817, CA-16359, and CA-19757 from the National Cancer Institute.

[2] Recipient of a National Cancer Institute Postdoctoral Fellowship.

[3] Scholar of the Leukemia Society of America.

[4] Abbreviations: DMSO, dimethylsulfoxide; FLC, Friend leukemia cells.

0006-291X/79/041281-07$01.00/0

It has been established that sialic acid residues[5] are the major ionogenic moieties at the external surface membrane of human erythrocytes (12). External cell surface sialoglycoconjugates have been implicated in many important biological phenomena (for an appropriate review see ref. 13) which include: masking of cell surface antigens; receptors for various lectins, virus particles, mycoplasma, hormones, and antibodies; transformation from normal to neoplastic cells; invasiveness and metastasis; and cell-cell interactions such as cellular adhesion, intercellular aggregation, and agglutination. In view of the potential significance of cell surface charge and sialic acid, we have examined these properties during erythrodifferentiation of FLC.

METHODS:

Tissue Culture and Induction Conditions. Clone 745 FLC cells were kindly donated by Dr. N. Gabelman of Mount Sinai School of Medicine, New York. FLC cells were passed three times weekly at 10^5 cells/ml in Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY) supplemented with 50 units/ml of penicillin, 50 µg/ml of streptomycin, 2 mM L-glutamine, and 15% (v/v) fetal calf serum (GIBCO). Cell cultures were incubated at 37°C in a humidified atmosphere of 10% CO_2 in air. Cell numbers were measured using a Coulter Model ZBI electronic particle counter. DMSO treatment was carried out in the same medium by addition of 1.5% DMSO (v/v, Burdick and Jackson Laboratories, Muskegon, MI) to exponentially growing FLC reseeded at 10^5 cells/ml. The proportion of differentiated cells in control and DMSO-treated cultures was measured using a benzidine peroxide cytological stain (14), modified so that the final benzidine (p-diaminodiphenyl dihydrochloride, Sigma Chemical Co., St. Louis, MO) concentration was 0.1%.

Electrophoretic Mobility. Saline-sorbitol medium of low ionic strength consisted of 4.5% (w/v) sorbitol, 0.0145 M NaCl and 0.3 mM $NaHCO_3$ (pH 7.2). Electrophoretic mobility measurements were made at 25 ± 0.1° in a horizontal cylindrical chamber of small volume (15). The chamber (Rank Brothers, Bottisham, Cambs., U.K.) was viewed by dark field illumination. Mobilities of the particles were calculated in $\mu m \cdot s^{-1} \cdot volt^{-1} \cdot cm$; each independent value was obtained by timing the movement of at least 38 particles with a reversal of polarity after each measurement. Unless otherwise noted, the data presented are from single experiments that are representative of at least four independent observations (i.e., each observation included 38 or more independent measurements). Alignment of the apparatus was checked by the method of Seaman (15). For calibration purposes, determinations of the mobility of washed human erythrocytes were made and compared to the value of -2.78 ± 0.08 $\mu m \cdot s^{-1} \cdot volt^{-1} \cdot cm$ reported by Seaman (15). Mobilities obtained in the low ionic strength saline-sorbitol medium were corrected to the viscosity of standard saline at 25°C with an Ostwald bulb viscometer.

---

[5] The term sialic acid is employed to indicate a family of N-acetylated neuraminic acids, the most common of which is N-acetylneuraminic acid (NANA). Specific reference to NANA has been made when appropriate.

Neuraminidase Treatment of FLC. Control and DMSO-treated FLC at a concentration of 10^7 cells/ml were incubated with varying concentrations of neuraminidase (EC 3.2.1.18; 28 U/mg, where one unit caused the release of one μmol of sialic acid per minute from bovine submaxillary mucin at 37°C and pH 5.0) derived from Clostridium perfringens (Worthington Biochemicals Corp., Freehold, NJ) in medium minus fetal calf serum for 15 minutes at 37°C. Following incubation with enzyme, the cells were washed four times with saline-sorbitol solution prior to electrophoretic mobility measurements. The intactness of cellular membranes was determined by trypan blue exclusion. Neuraminidase treatment at the concentrations and conditions employed did not result in a decrease in dye exclusion in either control or DMSO-treated cells.

Determination of sialic acid. Sialic acid content was determined using the periodate-resorcinol method of Jourdian et al. (16). Cells were collected and washed three times with buffer (7.40 g NaCl, 1.93 g $Na_2HPO_4 \cdot 7H_2O$, 0.39 g $NaH_2PO_4 \cdot H_2O$, pH 7.2). Periodate oxidation was carried out at 37°C for 60 min (conditions which destroy free sialic acid) to determine glycosidically bound sialic acid. Following reaction with resorcinol and extraction with t-butyl alcohol, the samples were centrifuged at 10,000 x g for 5 min to pellet biological debris. Periodate oxidation of N-acetylneuraminic acid (Sigma Chemical Company, St. Louis, MO) employed as a standard was carried out at 0° for 15 min.

RESULTS:

The growth and extent of differentiation of FLC under the conditions employed are shown in Figure 1. Control and DMSO-treated cells, seeded on day 0 at 10^5 cells/ml from exponentially growing stock cultures, replicated at approximately the same rate, doubling about every 12 hr. FLC treated in culture with 1.5% DMSO began accumulating hemoglobin, as measured by the percentage of benzidine-positive cells, by 72 hr and reached a maximum number of hemoglobin-containing cells by day 5. Populations of untreated FLC contained fewer than 1% hemoglobin-containing cells.

The electrophoretic mobilities of DMSO-treated and untreated control FLC are shown in Figure 2. A significant reduction in the mobility of DMSO-treated populations was observed beginning on day 2 of exposure to 1.5% DMSO and continuing through the remainder of the induction process, while no significant changes occurred in untreated control cells. The effect of neuraminidase treatment on electrophoretic mobility is shown in Figure 3. The data indicate that treatment with increasing concentrations of neuraminidase caused a dose-dependent decrease in the mobility of both control and DMSO-treated cells. Neuraminidase treatment of 3, 4, and 5 day control and DMSO-treated cultures resulted in mobilities of similar magnitude.

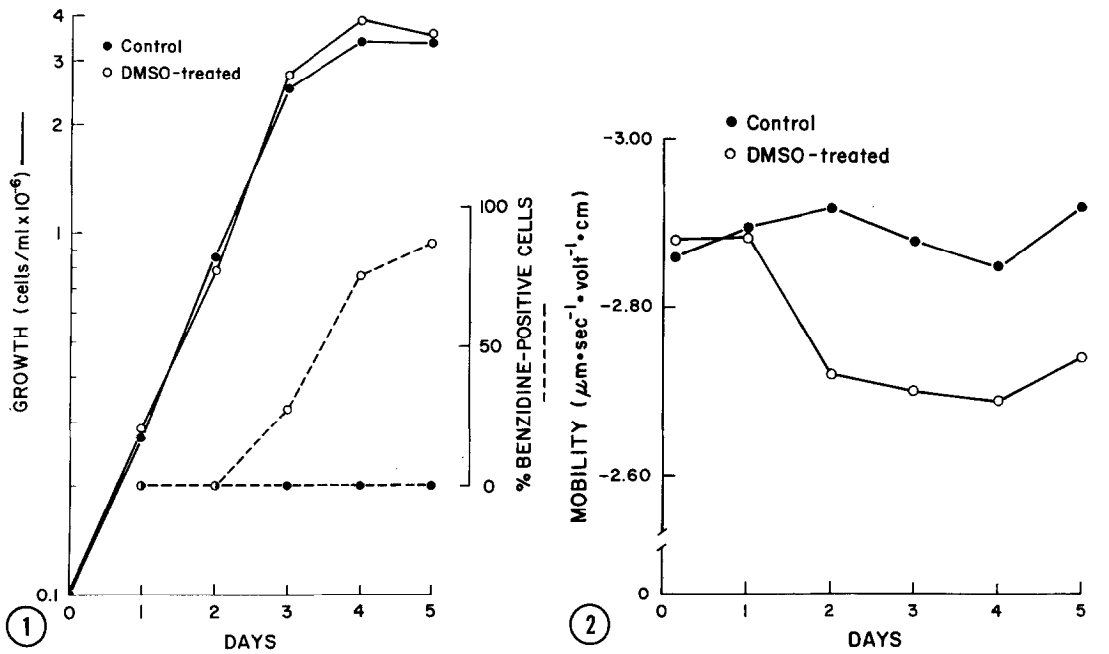


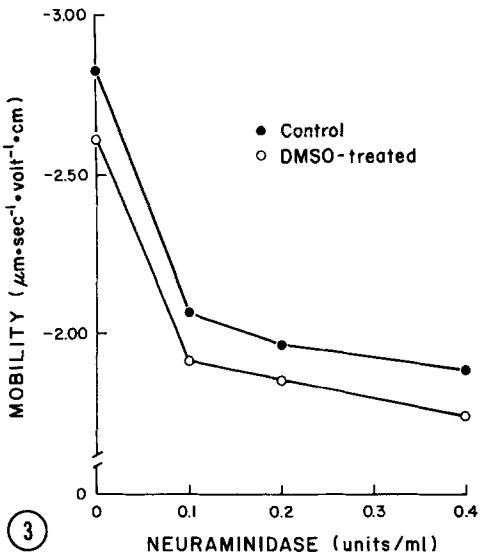
Fig. 1. Growth and differentiation of control and DMSO-treated FLC. Control and DMSO-treated (1.5%, v/v) cells were seeded on day 0 at 10^5 cells/ml from exponentially growing stock cultures. Cell numbers were obtained using an electronic particle counter and the % benzidine-positive cells was determined using benzidine peroxide as described in the Methods section.

Fig. 2. Electrophoretic mobility of control and DMSO-treated FLC. Control and DMSO-treated cells were prepared as described in Fig. 1 and mobilities were measured in low ionic strength saline-sorbitol medium. Each value was obtained by timing the movement of at least 38 cells with a reverse of polarity after each measurement. The data presented are representative of results from one of four independent experiments.

Glycosidically linked sialic acid was determined in control and DMSO-treated cells and the results are shown in Figure 4. DMSO-treated populations contained significantly less sialic acid per cell than control cultures after 4 and 5 days of exposure to the polar solvent.

DISCUSSION:

Interpretation of cell electrophoretic mobility is complex; however, with appropriately controlled conditions it can be used to make qualitative comparisons of the net surface charge of whole cells [these conditions and assumptions have been thoroughly discussed by Seaman (15)]. For cells in the size range



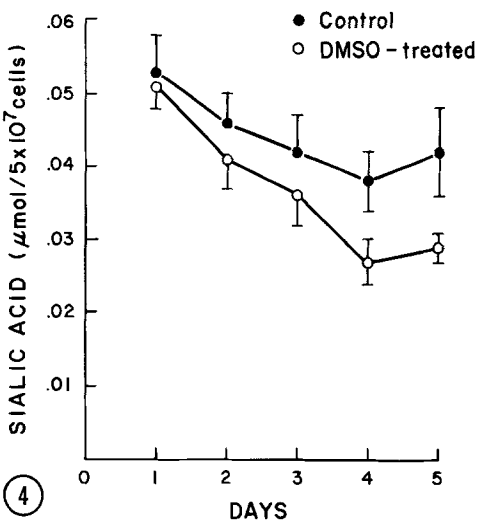


Fig. 3. Electrophoretic mobility of control and DMSO-treated FLC after treatment with neuraminidase. Control and DMSO-treated cells seeded on day 0 at 10^5 cells/ml were collected on day 2 and incubated with the indicated amount of neuraminidase for 15 minutes at 37°C; mobilities were determined as described in the Methods section.

Fig. 4. Sialic acid content of control and DMSO-treated FLC. Cells were collected at the indicated times and macromolecular bound sialic acid was determined. The results are the mean ± standard deviation of 4 samples. Values for control and DMSO-treated FLC were significantly different for days 4 and 5 ($p < 0.01$ by Students' t-test).

of 7-12 μ diameter under the low ionic strength conditions employed in this study, electrophoretic mobility appears to be an expression of the number of exposed ionogenic groups at the cell surface. Furthermore, under these conditions, alterations in cell shape or size in the absence of changes in the net surface charge do not affect the measurement of mobility (15). We have observed (Fig. 2), beginning as early as 24 hr after exposure to DMSO and continuing through the remainder of the induction period, a reduction in the negative electrophoretic mobility of DMSO-treated cells. This observation is interpreted to indicate that DMSO-treated cells have a decrease in net negative surface charge that coincides temporally with erythrodifferentiation. DMSO-treated FLC begin to accumulate hemoglobin at approximately 72 hr after exposure to the inducer; however, other indicies of the erythrodifferentiation process, such

as the appearance of globin mRNA (4), the appearance of erythrocyte membrane specific antigens (5) and the increase in the rate of synthesis and accumulation of spectrin (11), occur as early as 24 hr after addition of DMSO.

For many cell types, particularly erythrocytes, neuraminidase has been used to demonstrate the importance of sialic acid residues to net negative surface charge (17). The results in Figure 3 indicate that surface sialic acid residues, susceptible to removal by neuraminidase, contribute significantly to the net negative charge of both control and DMSO-treated FLC. Evidence that the decrease in net negative surface charge of DMSO-treated cells is due to a reduction in surface sialic acid content is presented in Figure 4. In these experiments, total cellular glycosidically bound sialic acid was measured and assumed to be a reflection of the amount of sialic acid on the cell surface. Since DMSO-treated cells progressively decrease in volume during the induction period (18), it is possible that the total surface area decreases and the number of sialic acid containing macromolecules on the cell surface decreases proportionately, which could account for the observed decrease in electrophoretic mobility and the lowering of the sialic acid content. In addition to changes in cell volume, alterations in surface architecture, such as a decrease in the number of microvilli on DMSO-treated FLC have been reported (5,10), which may be relevant to the present findings.

REFERENCES:

1. Friend, C., Scher, W., Holland, J. G., and Sato, T. (1971) Proc. Natl. Acad. Sci. U.S.A. 68, 378-382.
2. Preisler, H.D., Lutton, J.D., Giladi, M., Goldstein, K., and Zanjani, E.D. (1975) Life Sci. 16, 1241-1252.
3. Gusella, J., Geller, R., Clarke, B., Weeks, V., and Housman, D. (1976) Cell 9, 221-229.
4. Harrison, P.R. (1977) in Biochemistry of Cell Differentiation II, Vol. 15 (ed. J. Paul), pp. 227-267, University Park Press, Baltimore.
5. Ikawa, Y., Ross, J., Leder, O., Gielen, J., Packman, S., Ebert, P., Hayashi, K., and Sugano, H. (1973) in Differentiation and Control of Malignancy of Tumor Cells (eds. W. Nakahara, T. Ono, T. Sugimura, and H. Sugano), pp. 515-546, University Park Press, Baltimore.
6. Arndt-Jovin, D. J., Ostertag, W., Eisen, H., and Jovin, T. M. (1976) in Modern Trends in Human Leukemia II (eds. R. Neth, R. C. Gallo, K. Mannweiler, and W. C. Maloney), pp. 137-150, J. F. Lehmanns, Munich.

7. Germinario, R. J., Kleiman, L., Peters, S., and Oliveira, M. (1977) Exptl. Cell Res. 110, 375-385.
8. Mager, D., and Bernstein, A. (1978) J. Cell Physiol. 94, 275-286.
9. Eisen, H., Nasi, S., Georgopoulos, C. P., Arndt-Jovin, D., and Ostertag, W. (1977) Cell 10, 689-695.
10. Tsiftsoglou, A. S., and Sartorelli, A. C. (1977) Fed. Proc. 36, 886.
11. Eisen, H., Bach, R., and Emery, R. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 3898-3902.
12. Cook, G. M. W., Heard, D. H., and Seaman, G. V. F. (1961) Nature 191, 44-47.
13. Jeanloz, R. W., and Codington, J. F. (1976) in Biological Roles of Sialic Acid (eds. A. Rosenberg and C. Schengrund), pp. 201-238, Plenum Press, New York.
14. Orkin, S. H., Harosi, F. I., and Leder, P. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 98-102.
15. Seaman, G. V. F. (1975) in The Red Blood Cell II (ed. D. M. Surgenor), pp. 1135-1229, Academic Press, New York.
16. Jourdian, G. W., Dean, L., and Roseman, S. (1971) J. Biol. Chem. 246, 430-435.
17. Cook, G. M. W., and Stoddard, R. W. (1973) Surface Carbohydrates of the Eukaryotic Cell, pp. 56-97, Academic Press, New York.
18. Loritz, F., Bernstein, A., and Miller, R. G. (1977) J. Cell Physiol. 90, 423-438.