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Sialic Acid on Leukemia Cells: Relation to Morphology and Tumor Immunity (37896)

RICHARD C. REED, JORDAN U. GUTTERMAN, GIORA M. MAVLIGIT, AND EVAN M. HERSH

Department of Developmental Therapeutics, The University of Texas at Houston, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025

The lymphoblastic and myeloblastic subclasses of human acute leukemia differ in some parameters of host–tumor relationship. Patients with acute lymphoblastic leukemia (ALL) have a greater tendency for dissemination to extramedullary sites (1), and a weaker tumor-directed immune reactivity compared to patients with acute myelogenous leukemia (AML) (2, 3). These differences may be related to the glycoprotein coat of the leukemic cells, and more specifically, to its sialic acid moiety. The presence and concentration of these substances appear to regulate the electrophoretic mobility (4) and antigenicity or immunogenicity of these tumor cells (5–8). To further characterize the nature of the differences between various subclasses of leukemic blasts, the amount of sialic acid which can be removed from the cells by neuraminidase was correlated with tumor-directed immune reactivity in patients with acute leukemia.

Blast cells from 25 patients with AML and 10 patients with ALL were collected by leukapheresis as described previously (2, 3). Red blood cells were lysed by washing the cells two or three times in 40 ml of Tris-buffered ammonium chloride (9). Normal donor mononuclear cells were separated from defibrinated peripheral blood on a Ficoll–Hypaque gradient (10). Contaminating red cells were similarly lysed with Tris-buffered ammonium chloride. The resulting cell suspension consisted of 95% or more lymphocytes.

The blasts and normal lymphocytes were washed thrice with Eagle's minimal essential medium (MEM) and adjusted to a concentration of 5×10^7 cells per ml. One milliliter of cells was incubated with 50 units of Vibrio Cholera Neuraminidase (VCN) (General Biochemicals, Chagrin Falls, OH) at 37° for 60 min. Separate aliquots were also incubated with heat-inactivated VCN and buffer alone. Supernatants were analyzed for sialic acid by the thiobarbituric acid method of Warren (11).

To measure tumor-induced immune reactivity, lymphocyte blastogenesis was used. Peripheral blood lymphocytes were collected, washed, and cultured as described previously (2). Cultures contained 1×10^6 washed lymphocytes, 1 ml of serum (either autologous-drawn on the day of study, or normal allogeneic), and 2 ml of MEM. In addition to unstimulated controls, lymphocytes were stimulated with 5×10^5 or 10^6 autologous leukemic cells both unirradiated and irradiated with 1000 or 4000 R. Leukemic cells in similar doses and after similar treatment were also cultured alone and with irradiated lymphocytes. Cultures were incubated at 37° in an atmosphere of 5% CO_2 and air for 7 days. Harvesting was accomplished as described previously (2). During the last 3 hr of incubation, 2 μCi of [^{3}H]thymidine (sp act 1.9 Ci/mmole, Schwarz BioResearch) was added. The blastogenic responses were determined by the incorporation of the isotope into the acid-insoluble fraction as measured by liquid scintillation counting and expressed, after subtraction of appropriate controls, as the counts per minute per 10^6 lymphocytes. The stimulation index was defined as the num-

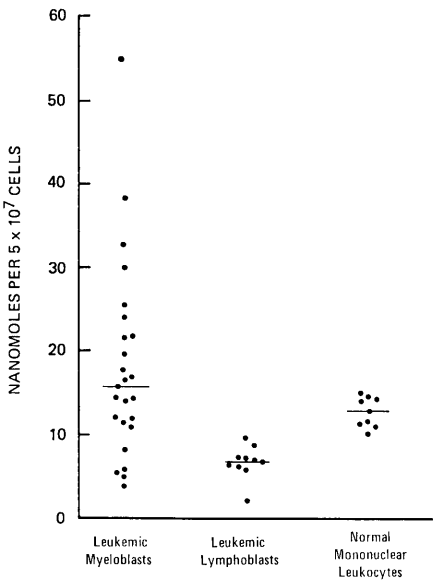

FIG. 1. Sialic acid removed by VCN from three types of cells. Horizontal lines indicate median values.

ber of counts per minute of a stimulated culture divided by the counts per minute of the appropriate unstimulated lymphocyte culture. A 200% increase was significant at the 99% level of confidence. Statistical analysis was performed with the chi-square and the Student t tests (12).

The releasable sialic acid content of leukemic blasts and normal donor mononuclear cells is shown in Fig. 1. Normal mononuclear leukocytes showed a mean removable sialic acid of 12.9 ± 0.6 nmoles/5 × 10^7 cells (median 12.9) with a narrow range. Leukemic (ALL) lymphoblasts, also showed a narrow range, but significantly lower levels of releasable sialic acid compared to mononuclear leukocytes (mean 6.9 ± 0.6, median 7.3 nmoles/5 × 10^7 cells; $P = 0.001$). The range of sialic acid values of ALL blasts did not overlap with the range in normal mononuclear leukocytes.

The amount of sialic acid released from leukemic myeloblasts showed a wide distribution. The mean and median values (18.3 ± 2.4, and 16.7 nmoles 5 × 10^7 cells) were significantly higher than the respective values for leukemic lymphoblasts ($P < 0.01$), but not significantly different from the values for normal cells ($P = 0.2$).

The relationship between the amount of sialic acid removed from different blasts and the frequency and degree of blastogenic responses induced by the unmodified blasts among autologous remission lymphocytes is shown in Table I. Patients with ALL, having uniformly low levels of removable sialic acid, showed low degree (median SI = 1.6) and low frequency (two of eight) of blastogenic responses. In contrast, patients with AML, showed a heterogenous pattern of blastogenic response with entirely different relationship to the sialic acid content on their blasts. Although the differences were not statistically significant, AML patients whose leukemic blasts carried less than 20 nmoles/5 × 10^7 cells of releasable sialic acid, tended to show higher frequency (8/9 vs 3/6) and more vigorous blastogenic responses (median SI = 13.4 vs 3.4) than patients whose leukemic blasts carried more than 20 nmoles of releasable sialic acid.

The difference between ALL and AML

TABLE I. Correlation Between Removable Sialic Acid and Tumor-Induced Lymphocytes Stimulation.

	ALL	*AML*	
Removable sialic acid[a] (Range)	<10 (2.4–9.7)	<20 (4.1–15.8)	>20 (21.8–55.7)
Blastogenic response to blast cells	2/8	8/9	3/6
No. positive[b]/ no. studied			
Median stimulation index	1.6	13.4	3.4

[a] Nmoles/5 × 10^7 blasts.
[b] Stimulation index ≥3.

blasts in the amount of removable sialic acid, were not reflected in the serum levels of free sialic acid in these patients. Sera collected from normal donors, and from the patients at the time of blast cell collection (leukapheresis), showed comparable sialic acid levels (medians: AML 2.2; ALL 2.7; normal donors 1.8 nmoles/ml.

These results demonstrate the diversity of leukemic blasts in terms of surface sialic acid content and their immunobiological reactivity. The most striking finding was the homogeneity of ALL blasts with their uniformly low levels of surface sialic acid and their poor capacity to stimulate blastogenesis among remission lymphocytes. Similarly low levels of surface sialic acid have also been demonstrated on chronic lymphatic leukemia cells (13). Low levels of surface sialic acid could be due to shedding-off from the circulating leukemic cells (14). This is unlikely in view of the low serum levels of sialic acid. Another unlikely possibility is that the VCN may have been unable to remove the cell surface sialic acid from ALL blasts. The most likely possibility is the lack of synthesis of this substance by ALL blasts. This phenomenon has been described in other immature cells (15). A concurrent poor synthesis of tumor-associated antigen would explain the observation of poor *in vitro* stimulatory capacity of ALL blasts (2, 3) and would provide a suitable alternative to the current concept of masking of tumor antigen by sialic acid. It could also explain the frequent dissemination of ALL into extra-medullary sites (1). This is analogous to the observation that metastatic breast carcinoma cells but not primary carcinoma cells are devoid of cell-surface sialic acid. In fact, it has been suggested that sialic acid containing glycoprotein may be necessary for the antigenic expression of tumor cells (14). It is also conceivable that attempts at enzymatic removal of sialic acid from ALL blasts would not increase their immunogenicity or antigenicity.

In contrast to ALL, AML blasts showed a wide spectrum of removable sialic acid values with a tendency toward an inverse relationship between these values and the degree and frequency of the blastogenic responses induced by the unmodified blasts among autologous remission lymphocytes. This indicates that AML is probably a more heterogenous disease compared to ALL, and that low or high antigenicity in AML blasts may be influenced by more complex factors in addition to sialic acid coating. It is conceivable, however, that enzymatic removal of sialic acid may improve the antigenicity and immunogenicity of those AML blasts carrying high levels of this compound. Studies in this direction are now underway in our laboratory. Since various types of tumor cells differ in the amounts of sialic acid present on their surface, we suggest that the use of neuraminidase-treated tumor cells for specific immunotherapy should be preceded by a careful *in vitro* measurement of their releasable surface sialic acid.

Summary. Blast cells from 35 patients with adult acute leukemia and mononuclear cells from 10 normal donors were treated with neuraminidase and the amount of sialic acid released was measured. These values were correlated with the ability of the unmodified blasts to stimulate blastogenesis among autologous remission lymphocytes. Uniformly low amounts of sialic acid were released from leukemia lymphoblasts (10 patients). This was correlated with a consistently poor ability of these lymphoblasts to stimulate autologous lymphocytes. In contrast, a wider range of sialic acid levels were determined for leukemic myeloblasts (25 patients). Myeloblasts releasing excessively high amounts of sialic acid tended to stimulate autologous lymphocytes poorly compared to the vigorous stimulation evoked by myeloblasts releasing lower amounts. Similar studies of cell surface properties should facilitate the understanding of the immune response evoked by human tumor cells.

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