ORGANIZATIONAL DIFFERENCE OF CELL SURFACE "HEMATOSIDE" IN NORMAL AND VIRALLY TRANSFORMED CELLS*

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It has been suggested that cell surface carbohydrates may play some roles in determining specificities of intercellular interactions by forming "recognition site" on cell surface which is recognized by complementary sites on other cells or macromolecules (Vogel and Sachs, 1964: Gesner and Ginsburg, 1965; Cox and Gesner, 1965; Kalckar, 1965; Shen and Ginsburg, 1968). Malignant transformation of cells may well be a result of the change of such "recognition site" on cell surfaces. This idea has been supported by the presence of a specific carbohydrate on the surface of the transformed cells (Aub et al., 1963; Burger and Goldberg, 1967; Hakomori et al., 1967; Hakomori and Murakami, 1968).

It is known, however, some of the membrane-bound carbohydrates are not directly exposed on the cell surface ("Kryptoantigen") (Koscielak, et al.; Uhlenbruck, et al., 1968). Anti-"globoside" serum does not react with mature intact erythrocytes (Koscielak, et al., 1968) but does react with fetal erythrocytes although the quantity of globoside in mature and immature erythrocytes was in the same level (Hakomori, 1968). It was considered, therefore, that the specificity of cell surface could be determined not only by the quantity and the quality of glycolipids themselves, but also by the organizational state of glycolipids with other components of cell surfaces. Present communication reports that the normal and the virally transformed

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cells differ in regard to the quantity of glycolipids and the reactivity of cell surface "hematosides" to their specific antibodies.

METHODS - Ten cell lines (see Table I) were grown in a modified Eagle's medium (3X amino acids and 1.5X vitamines) supplemented with 20% fetal calf

Table I. Content of hematoside, lactosylceramide, and glucosylceramide in normal and malignant-transformed cell lines (in μg per 100 mg protein).

	"N-acetyl-hematoside"	"N-glycolyl-hematoside"	Lactosyl-ceramide	Glucosyl-ceramide
Mouse fibroblast "3T3" cells*	87	90	<5	15
Do., transformed by polyoma virus (3T3Py)*	45	37	<5	20
Do., transformed by SV40 virus (3T3SV)*	38	25	<5	-
Do., double transformed by polyoma and SV40 (3T3DT)†	12	15	<5	30
Hamster fibroblast BHK C13-B_2‡	550	<5	30	-
Do., BHK C13/21¶	300	<5	50	-
Do., transformed by Schmidt-Rupin type Rouse virus (RS)¶	60	<5	65	-
Do., transformed by Bryan type Rouse virus (BR)¶	75	<5	80	-
Do., transformed by polyoma virus (BHKPy)‡	95	<5	90	-
Human heteroploid fibroblast§	850	-	25	85
Do., trnasformed by SV40 virus§	85	-	15	120

* See, Todaro, Green, and Goldberg (1964); donated by Dr. P. Cox
† See, Todaro, Habel, and Green (1965); donated by Dr. G. Todaro
‡ Subcloned by Dr. W. T. Murakami, ¶ See, Vigier and Montagnier, donated by Dr. L. Montagnier, § See, Todaro, Wolman, Green (1963); donated by Dr. Pious

serum for human cell lines and 10% calf serum for others. The glycolipids were extracted, analyzed and characterized according to the procedure previously described (Hakomori and Murakami, 1968). The anti-hematoside sera were prepared according to the method for preparing anti-"globoside" serum (Koscielak et al., 1968). Two hematosides, one with N-acetylneuraminyl(2→3) galactosyl(1→4) glucosyl residue (I:"N-acetylhematoside") (Svennerholm, 1963), another with N-glycolylneuraminyl(2→3)galactosyl(1→4) glucosyl residue (II: "N-glycolylhematoside") (Yamakawa and Suzuki, 1951; Klenk and Padberg, 1962) were used as antigens. The reactivities of antisera, as tested by complement fixation, were highly specific to the residue I, and or residue II, respectively. The antisera were not reactive to mono-, and disialogangliosides. Immunochemical characterization of these antisera will be described elsewhere.

The reactivity of cell lines as described in Table I was tested by anti-hematoside. Cells were labelled by Cr^{51} directly, or after being trypsinized for 15 minutes, and the reactivity of the cells to the anti-hematoside was determined by the release of the isotope due to the immunolysis of the cells in the presence of complement (Wigzell, 1965).

RESULTS AND DISCUSSION - "N-acetylhematoside" was found in all "BHK" cells and human fibroblast as the principal glycolipid, while both "N-acetyl-", and "N-glycolyl-hematoside" were found in all "3T3" cells. Co-existence of "N-acetyl-", and "N-glycolyl-hematoside" has been shown for dog erythrocytes (Handa and Yamakawa, 1964). All the virally transformed cells, irrespective of the origin of cells and kind of tumorgenic virus, were characterized by a lower level of hematosides than the original normal cell lines (per protein content) (see Table I). Presence of lactosylceramide was limited to "BHK" and human cell lines and not seen in "3T3" cell lines, however, presence of glucosylceramide in "3T3" as well as human cell lines and its increase in the transformed cells were observed. The relative quantity of lactosylceramide or glucosylceramide to the hematoside of all the transformed bells increased. Thus the previous results that "incompleteness of carbohydrate chain" in the transformed cells (Hakomori and

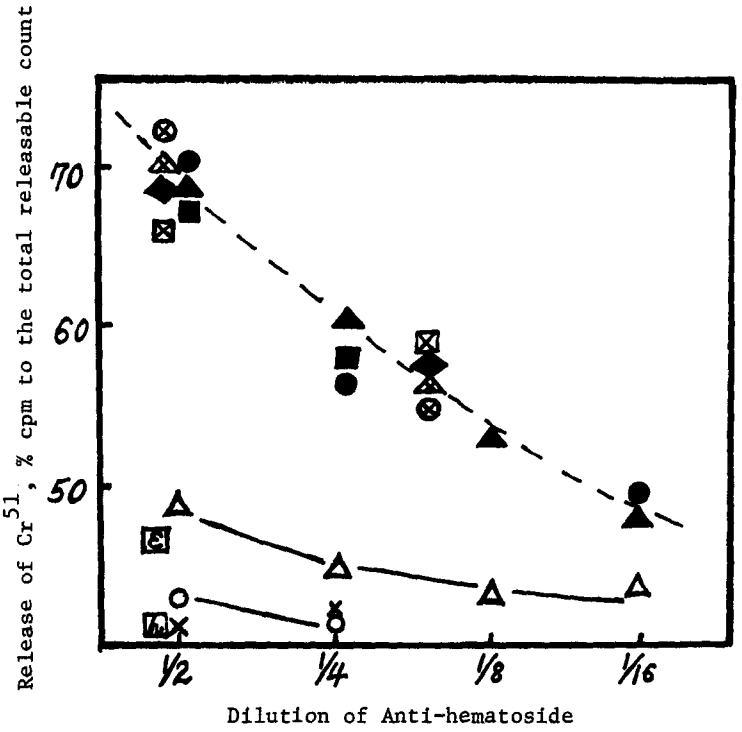

Fig. 1. Reactivities of cells in anti-hematoside antiserum

○ BHK; △ 3T3: ● 3T3-Py; ▲ 3T3 SV; ■ RB; ◆ BHK Py; ⊗ BHK, trypsinized; ⧋ 3T3, trypsinized; ⊠ RB, trypsinized, ⊡ RB, with 25 μg of N-acetylhematoside; ⊡ RB, with 25 μg of N-glycolylhematoside. All these activities were tested in anti-N-acetylhematoside. The antiserum showed highest fixation of complement at the dilution of 1:128 with 4-16 μg of hematoside, complexed with "carrier" lipid (Koscielak et al, 1968). ✕ 3T3 trypsinized tested in anti-N-glycolylhematoside. Control values by preimmune sera and C' were always lower than 40%. Reactions were carried out with 0.1 ml of antisera of different dilutions, 0.1 ml of C' (5X dilution of guinea pig sera), and 0.1 ml of cell suspension which contain 0.8×10^6 cells, or $7\text{-}10 \times 10^4$ c.p.m. radio activity. "Total releasable count" was the activity of supernatant when cells were lysed in 0.5% acetic acid.

Murakami, 1968) have been further extended and confirmed.

While total amount of hematoside in the transformed cells was significantly lower than the normal cells, the reactivity of hematoside to anti-hematoside serum was much higher in the transformed cells than that of normal cells (see Figure 1). The reactivity of cells to the anti-hematoside is considered to be proportional to the quantity of hematosides exposed to the cell surfaces, and was specifically inhibited by 25 μg (per 0.4 ml) of hematoside.

Treatment of normal cells with trypsin greatly enhanced their reactivity to anti-hematoside and the reactivity of the trypsinized normal cells was even slightly higher than that of the transformed cells. The reactivity of "3T3" cells to the anti-"N-glycolyl-hematoside" was very low while that of the transformed "3T3" cells reacted as high as that shown by anti-"N-acetyl-hematoside" serum. Hematosides were not detected in the trypsin-releasable moiety of either normal or malignant cells.

The results indicate that hematosides on normal cells are masked by some proteinous cover which is removable by trypsin, whereas hematosides of the transformed cells are unmasked. It has been known that the trypsin-releasable material from mammalian cell surface contains glycopeptides (Ambrose and Langley, 1965; Winzler et al., 1967; Shen and Ginsburg, 1968), whereas it was made clear,through this study,glycolipids are present not as trypsin releasable form, but are firmly bound to the cells.

The mechanism which masks or unmasks some cellular glycolipid must be a part of the regulatory mechanism which determines cell surface property. The "incompletion of the carbohydrate chain" and the incompletion of masking of these carbohydrates with proteineous cover provides the surface property of cells as being immature, and vice versa.

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