Cell Surface Galactosyltransferase and Lectin Agglutination of Thymus and Spleen Lymphocytes  
(cell membrane/blast transformation)

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ABSTRACT Lectin agglutination and cell surface galactosyltransferase (EC 2.4.1.67; 1-O-a-D-galactosyl-myoinositol:raffinose galactosyltransferase) enzyme activity have been studied with thymus and spleen lymphocytes of neonatal rats. Thymus lymphocytes were more agglutinable by concanavalin A than by wheat germ agglutinin, whereas spleen lymphocytes were more agglutinable by wheat germ agglutinin than by concanavalin A. Thymus lymphocytes, but not spleen lymphocytes, of neonatal rats could be blast transformed by concanavalin A. Cell surface galactosyltransferase activity was present on both types of lymphocytes, but was greatly increased on thymus cells after blast transformation. The differences in lectin agglutination suggest a difference in the surface membranes of thymus and spleen lymphocytes. The increase in cell surface galactosyltransferase activity with blast transformation of thymus lymphocytes may be related to the exteriorization of the Golgi apparatus into the plasma membrane.

The cell surfaces of T (thymus derived) and B (bursa equivalent derived) lymphocytes are known to differ in a number of important respects, depending on the species. T cells of mice possess theta antigen (1) and have a high surface charge (2); human T lymphocytes form rosettes with sheep erythrocytes (3). B cells have immunoglobulins on the cell surface (4), receptors for complement C3 (5), and possess other antigens not found on T cells. On the other hand, T cells can be blast transformed by concanavalin A (Con A), a property not shared by B cells (6).

A recent study from this laboratory showed a correlation between Con A agglutination of various cells and the activity of cell surface galactosyltransferase (EC 2.4.1.67; 1-O-a-D-galactosyl-myoinositol:raffinose galactosyltransferase) (7). Agglutinable cells, such as rabbit erythrocytes, rat intestinal crypt cells, and TA ST ascites cells had significantly higher surface galactosyltransferase activity than nonagglutinable cells, such as human erythrocytes, rat intestinal villus cells, or TA H ascites cells. Furthermore, purified galactosyltransferase from rabbit erythrocyte membrane (which formed a precipitate in agarose gel with Con A), when adsorbed to human erythrocytes, produced Con A agglutination of previously nonagglutinatable human erythrocytes. Thus, in several cell types there appears to be a correlation of plasma membrane galactosyltransferase with both the degree of cell differentiation and susceptibility to Con A agglutination.

In this study we demonstrate that T lymphocytes of neonatal rats were preferentially agglutinated and blast transformed by Con A, while spleen lymphocytes (S cells) were agglutinated by wheat germ agglutinin (WGA) and not blast transformed by Con A. Cell surface galactosyltransferase was present in both lymphocyte populations, but was markedly increased only on T cells as a result of blast transformation.

MATERIALS AND METHODS

Preparation of Cells. Thymus and spleen lymphocytes were prepared from 5- to 6-day-old neonatal or adult female Sprague-Dawley rats (Charles River Laboratories, N. Wilmington, Mass.). The thymus and spleen were removed and minced into approximately 1-mm³ pieces. The cells were separated from the stroma by gentle pipetting and subsequent filtering through cotton gauze. The cells were washed three times with phosphate-buffered saline (pH 7.4), and erythrocytes were removed by ammonium chloride lysis (8). For agglutination experiments, the cells were resuspended at 10⁶/ml in phosphate-buffered saline (pH 7.4); for cell surface galactosyltransferase activity they were resuspended in a buffer containing 0.1 M sodium cacodylate and 0.154 M NaCl, pH 7.4 at 10⁶-10⁷ cells/ml.

Lectin Agglutination. Incubation mixtures contained 0.05 ml of a cell suspension of T or S cells and 0.05 ml of Con A or WGA at various concentrations. Degree of agglutination (0 to ++++) was determined after a 30-min incubation in a 37° water bath, as described (7). Control tubes for agglutination assays consisted of cells and equal volumes of buffer without lectin. Con A was obtained as a thrice-crystallized powder from Miles-Yeda, Ltd. (Kankakee, Ill.). WGA was purified from wheat germ acid phosphatase (Miles-Yeda) by the method of Burger and Goldberg (9).

Galactosyltransferase Assay. The incubation mixture contained: 0.05 ml of lymphocyte suspension in NaCl-cacodylate buffer (pH 7.4), 0.39 nanomoles of UDP-[14C]galactose (257 mCi/mmol, New England Nuclear Corp.), 0.5 μM MnCl₂, and 250 μg of fetuin acceptor (20 mg/ml in 0.15 M NaCl) in a final volume of 72.5 μl. Fetuin acceptor (fetuin minus terminal sialic acid and penultimate galactose) was prepared by the method of Kim et al. (10). Incubation was for 30 min at 37°, and the reaction was terminated by the addition of 4 ml of cold 5% phosphotungstic acid in 2 M HCl. The precipitated proteins were collected on 2.4-cm
Cell Surface Galactosyltransferase of Lymphocytes

Fig. 1. Effect of Con A and WGA on the agglutination of thymus (T) and spleen (S) cells of neonatal rats.


Cell Surface Galactosyltransferase of Lymphocytes

RESULTS

Lectin Agglutination. Fig. 1 compares the agglutination of T- and S-derived lymphocytes by Con A and WGA. The T-cell population was preferentially agglutinated by Con A. S cells were agglutinated by WGA, and to a lesser extent by Con A, but at very high concentrations there was some agglutination by both lectins. At all Con A concentrations tested, the agglutination of T cells by Con A completely reversed by 0.1 M alpha-methylmannoside and agglutination of S cells by WGA, was reversed by 0.1 M N-acetylglucosamine.

Blast Transformation. As an indication of lymphocyte blast transformation, the incorporation of [3H]thymidine into cellular DNA was measured. In the presence of Con A there was stimulation of [3H]thymidine incorporation into T, but not S cells. As shown in Fig. 2, there was a sharp concentration optimum with maximum incorporation occurring at 5 μg of Con A per ml. Spleen cells did not undergo blast transformation with Con A at concentrations ranging from 0.5 to 10 μg/ml. In contrast to neonatal lymphocytes, the lymphocytes from adult thymus and spleen were both stimulated by 5 μg/ml of Con A, leading to an 8- to 10-fold increase in [3H]-thymidine incorporation above control cultures.
After incubation with \[^3H\]thymidine, radioautography of neonatal T and S cells was performed to determine if there was a difference in DNA-synthesizing capacity between these two cell populations. The mean percentage of nuclear labeling was 10.9% for T cells, and 16.5% for S cells. Thus, the lymphocytes were comparable in the relative proportion of cells in the resting phase capable of lectin stimulation.

Galactosyltransferase Activity. T and S lymphocytes were shown to possess surface galactosyltransferase activity with both endogenous and exogenous acceptor. The cell surface enzyme for both T and S lymphocytes had a pH optimum of 7.4, required Mn\(^{++}\) for optimum activity, and was specific for fetuin from which sialic acid and galactose had been removed. The activity with exogenous fetuin acceptor was 3- to 4-fold greater than with endogenous cell surface acceptor for both T and S cells. In order to demonstrate that the enzyme activity remained membrane-bound and was not released into the medium during the assay, T and S cells were preincubated in RPMI medium for 2 hr and then harvested by centrifugation. Greater than 95% of the enzyme activity remained with the cells, indicating that the enzyme was membrane-bound and not released into the medium under these conditions.

In our previous studies, which suggested a correlation between cell surface galactosyltransferase and Con A agglutinability (7), it was shown that galactosyltransferase activity was invariably present on the surface of agglutinable cells, and was generally higher in agglutinable than in nonagglutinable cells. With lymphocytes, on the other hand, the specific activity of galactosyltransferase was higher in the nonagglutinable S cells when expressed as cpm/mg of protein or cpm/10\(^6\) cells (Table 1). However, when enzyme activity was expressed on the basis of cell surface area, the T cells had a somewhat higher enzyme activity per cm\(^2\) of surface area.

Galactosyltransferase activity was also determined on T and S lymphocytes during blast transformation induced by Con A. As shown in Fig. 3, the transferase activity on T cells rose from very low pretreatment levels to 1970 cpm/10\(^7\) cells 72 hr after stimulation with 5 \(\mu\)g/ml of Con A. In control T cell cultures that were not blast transformed there was a negligible increase of galactosyltransferase activity from 2 to 72 hr. S cells showed only minimal changes in galactosyltransferase activity over 72 hr, either with or without Con A. Con A appeared to cause a slight depression of surface galactosyltransferase activity in S cells. We have noted a depression of transferase activity by Con A with a variety of other cells, and have interpreted this as supporting the hypothesis that Con A binds to the enzyme, the acceptor, or to both (7).

**DISCUSSION**

The present studies indicate that T and S lymphocytes of neonatal rate can be differentiated by their relative agglutination with Con A and WGA. T cells, but not S cells, were blast transformed by low concentrations of Con A, suggesting that the neonatal rate either has no T lymphocytes in the spleen or that the Con A receptor is not present on those T cells that may have peripheralized to the spleen at 7 days. In contrast, adult spleen lymphocytes could be blast transformed by Con A, suggesting that some T cells were present in the spleen in the adult animal.

Previous data from this laboratory demonstrated that galactosyltransferase activity in rat intestine is higher on the rapidly dividing crypt cell than on the nondoivding villus cell (11). We have also shown that cell surface galactosyltransferase appears to be associated with Con A agglutination, and that this transferase activity is often significantly greater on cells that are agglutinable compared to those that are nonagglutinable with Con A (7). In the current study, it was found that both T and S lymphocytes possess surface galactosyltransferase capable of transferring a galactose

**TABLE 1. Cell surface galactosyltransferase activity on T and S lymphocytes**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>cpm/10(^6) cells</th>
<th>cpm/mg of protein</th>
<th>cpm/cm(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>1520</td>
<td>4600</td>
<td>135</td>
</tr>
<tr>
<td>S</td>
<td>4800</td>
<td>10210</td>
<td>106</td>
</tr>
</tbody>
</table>

* Exogenous galactosyltransferase activity with fetuin acceptor was measured in T and S cells as in Materials and Methods. Mean cell diameter measured from photomicrographs was 6 \(\mu\)m for T cells and 12 \(\mu\)m for S cells.
residue to an exogenous fetuin acceptor or to endogenous glycoprotein acceptors. Surface galactosyltransferase requires Mn** for full activity and has a pH optimum of 7.4. Similar properties have been demonstrated for a number of membrane-bound glycosyltransferases from a variety of sources (12-14).

The evidence that the enzyme is located on the external surface of the plasma membrane is based on several observations. (1) The enzyme activity was demonstrated with intact, metabolically active lymphocytes that excluded Trypan blue and that could undergo blast transformation. (2) The exogenous fetuin acceptor became labeled and accumulated in the medium; it is unlikely that this highly charged molecule entered the cell, became glycosylated, and was then reexcreted into the medium. (3) The enzyme was not released from the lymphocyte surface into the medium during 2 hr of preincubation. (4) The enzyme is particulate and requires detergent treatment or extensive sonication for solubilization (J. T. LaMont, unpublished observations).

We have also shown a marked increase of cell surface galactosyltransferase activity on blast-transformed T lymphocytes as compared to resting T lymphocytes. This observation is analogous to those on rat intestinal cells, where it has been shown that the actively dividing crypt cells have high cell surface galactosyltransferase activity while mature nondividing villus cells have much lower transferase-specific activity (11). Galactosyltransferase activity has been localized to the Golgi apparatus and smooth endoplasmic reticulum in intestine (10), liver (14), and salivary gland (15). It has been suggested by Roseman (16), Keenan and Morre (12), and Weiser (11) that newly synthesized plasma membrane originates from the Golgi apparatus and smooth endoplasmic reticulum and is then exteriorized during the S and G2 phase, just prior to cell division. Therefore, one would expect that blast transformation would be accompanied by synthesis of new surface membrane and appearance of Golgi-associated enzymes such as galactosyltransferase, as has been suggested for mitotically active intestinal crypt cells (11).

The role of cell surface galactosyltransferase activity in the function of lymphocytes is currently unknown. Roseman, on the basis of studies with neural retinal cells, has suggested that glycosyltransferases play an essential role in cell-to-cell adhesion (16). A similar role has been assigned to platelet membrane galactosyl- and glucosyltransferase during specific aggregation of platelets to collagen (13, 17). A possible role of galactosyltransferase in contact inhibition of growth in 3T3 cells was suggested by Roth and White (18). Bosmann and his colleagues have shown a correlation between high surface glycosyltransferases and an increased tendency of tumor cells to metastasize (19). Our data with rat lymphocytes are most consistent with the hypothesis that the level of galactosyltransferase activity on the plasma membrane is correlated with entry of the cell into S phase in preparation for cell division. A similar observation on cultured mouse lymphoma cells has recently been made by Bosmann (20). Thomas has shown that cell surface expression of human blood group B antigen is markedly increased in phytohemagglutinin-stimulated murine lymphocytes as compared to resting lymphocytes (21). This observation may be related to our finding of increased cell surface galactosyltransferase in blast-transformed cells, inasmuch as blood group B antigenicity is conferred by a terminal α-galactosyl residue.

Our previous experiments with rabbit erythrocyte plasma membranes suggested that galactosyltransferase enzyme was directly involved in Con A agglutination of these cells (7). It is evident that the level of galactosyltransferase activity is not the only important parameter in Con A agglutination, inasmuch as the relatively less agglutinable S cells had significantly higher galactosyltransferase activity per cell than T cells, although the more agglutinable T cells had higher activity per cm² of surface membrane. Thus, there still appears to be a correlation between Con A agglutination and the presence of galactosyltransferase activity. Con A agglutination of rabbit erythrocytes is dependent upon a terminal α-mannose moiety of the cell surface galactosyltransferase (D. Podolsky and M. Weiser, unpublished observations). It is possible that there are several isoenzymes of galactosyltransferase on the surface of lymphocytes and that it is the Con A-binding enzyme that has a terminal mannose residue.

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