Different Effects of Concanavalin A and E-Phytohemagglutinin upon Lymphocyte Glycosyltransferase Activities

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INTRODUCTION

Stimulation of lymphocytes in vitro by mitogenic plant lectins has been used to study mechanisms of lymphocyte activation and proliferation (30). During mitogenic stimulation lectin binds to specific cell surface receptors initiating a complex series of biochemical and morphological events that may culminate in cell division and proliferation (30). These events may be accompanied by a variety of alterations in the content and/or exposure of complex saccharide structures at the lymphocyte surface detected by changes in antigen and receptor patterns of transformed versus resting lymphocytes (4, 20, 24, 27). Recently, Gahmberg et al. (7) have demonstrated such changes directly in resting and lectin-activated mouse lymphocytes labeled by the galactose oxidase-sodium borotritide method. Increasing evidence implicates surface saccharide components as important determinants of the social behavior of cells participating in such important phenomena as embryonic differentiation, cell proliferation, neoplastic transformation, and immunological responsiveness. Such data provide the possibility that complex alterations in lymphocyte membrane carbohydrate structure during activation of these cells may be intimately related to and indeed necessary for both the actual process of mitogenesis and subsequent functional capabilities of the transformed cells.

Recent studies in our laboratory have demonstrated that human lymphocytes transformed with either E-PHA³ or Con A developed different complements of cell surface oligosaccharides as detected by lectin-binding experiments (24). Furthermore, the data indicated that these structural alterations were mediated by different mechanisms in lymphocytes transformed by these 2 plant lectins. De novo protein synthesis was required for the cell surface changes associated with E-PHA stimulation but not for those changes induced by Con A which appeared to stimulate attachment of saccharide structures to presynthesized membrane proteins (24).

Current evidence suggests that the synthesis of the terminal carbohydrate portions of certain glycoproteins and glycolipids is under the control of a multiglycosyltransferase system of enzymes (21). These transferase enzymes catalyze the stepwise addition of monosaccharide units from nucleotide sugar donors to incomplete carbohydrate chains attached to protein or lipid (21). Because of the different alterations induced in lymphocyte membrane saccharide structure and biosynthesis by E-PHA or Con A, we have examined glycosyltransferase activities in lymphocytes stimulated with these mitogens and compared the results with activities found in resting lymphocytes. The results indicate that E-PHA and Con A have different effects on the synthesis of complex saccharides by cultured human lymphocytes, and they suggest that despite their morphological similarities, lymphocytes transformed with different plant mitogens may express a spectrum of biochemical and functional capabilities.

MATERIALS AND METHODS

Lymphocyte Preparation

Lymphocytes used in all experiments were obtained from normal human volunteers. Mononuclear cells were isolated from defibrinated peripheral venous blood by dextran sedimentation followed by isopyknic centrifugation (2). Ninety to 95% of these cells were peroxidase negative, and trypan blue exclusion was usually greater than 95%.

Lectins

E-PHA was prepared from phytohemagglutinin P (Difco

² The abbreviations used are: E-PHA, E- phytohemagglutinin; Con A, concanavalin A; PTA, phosphotungstic acid.

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Received March 20, 1978; accepted September 18, 1978.
Lectins and Lymphocyte Glycosyltransferase Activity

Lymphocyte Incubation Studies

Lymphocytes, 2 × 10^6, were incubated in 16- × 125-mm polystyrene culture tubes (Falcon Plastics, Oxnard, Calif.) at 37° in a humid atmosphere of 5% CO_2-95% air in 2 ml of Medium 199 containing 12.5% heat-inactivated (56°; 30 min) fetal calf serum, 2% 200 mM L-glutamine, and 1% penicillin-streptomycin solution (all from Microbiological Associates, Bethesda, Md.). Plant mitogens were added in concentrations shown to produce optimal stimulation of [3H]thymidine incorporation in separate dose response experiments. These concentrations were: E-PHA, 5 μg/ml; Con A, 50 μg/ml. All incubations were carried out for 72 hr, and cell viability as judged by trypan blue exclusion was always greater than 90% at 72 hr.

In some experiments puromycin (Calbiochem, San Diego, Calif.), 0.5 μg/ml, was present during the 72-hr incubations. For the assay of the effect of puromycin on lymphocyte protein synthesis, cells grown for 68 hr were pulse labeled for an additional 4 hr with 2.5 μCi of [3H]leucine (250 mCi/ml; Amersham/Searle), and added to 10 ml Hydromix Scintillation Cocktail (Yorktown Research, Inc., S. Hackensack, N. J.) for counting in a Beckman LS-345 Liquid Scintillation System (Beckman Instruments, Inc., Fullerton, Calif.).

Removal of Bound Lectin

Prior to the use of transformed lymphocytes in glycosyltransferase assays, residual lectin remaining bound to the cell surface after the 72-hr incubation was removed as previously described (24). Experiments with radiolabeled lectins during the culture period indicated that less than 0.1 μg of lectin remained bound per 5 × 10^6 cells following these procedures (24).

Glycosyltransferase Assays

Glycoprotein Acceptors. For preparation of asialofetuin, 1 g fetuin was dissolved in 100 ml 0.05 N H_2SO_4 and incubated for 1 hr at 80° (25). The mixture was neutralized with NaOH to a pH of 7.2 and extensively dialyzed. Analysis of the desialized fetuin for residual sialic acid by the method of Warren (28) demonstrated that 90 to 95% of the sialic acid was removed from native fetuin by this procedure. Asialoagalactofetuin was prepared from asialofetuin by periodate oxidation followed by sodium borohydride reduction and mild acid hydrolysis (26). As previously described this procedure resulted in release of approximately 80 to 90% of galactose residues from asialofetuin (1). N-Acetylglucosamine was removed from ovalbumin by incubation with β-N-acetylhexosaminidase prepared from jack bean meal by the method of Li and Li (13). Ovalbumin, 1 g, was dissolved in 100 ml 0.05 N sodium citrate buffer, pH 5.0, containing β-N-acetylhexosaminidase, 12.5 units/ml. Incubation was carried out at 37° under toluene for 72 hr. The enzyme was inactivated by heating, the preparation was centrifuged to remove precipitated material, and the supernatant was retained and dialyzed. Gas chromatographic analysis (kindly performed by Dr. G. Pier) indicated that the derivative compound retained only 12% of the N-acetylglucosamine but all of the mannoside of the parent compound.

Enzyme Activity. Assays for glycosyltransferase activities in whole cells were based upon a modification of the method of Lamont et al. (12). Reaction mixtures for determination of glycosyltransferase activity contained the following components in 335 μl of 0.1 mM sodium cacodylate, pH 7.4-0.15 M NaCl: 4 to 5 × 10^6 lymphocytes, 1.5 μmol MgCl_2, 1.5 μmol MnSO_4, 0.4 nmol of either CMP-[3H]sialic acid (specific activity, 196 μCi/mm; Amersham/Searle), UDP-[3H]galactose (specific activity, 196 μCi/mm; Amersham/Searle), or UDP-N-[3H]acetyl glucosamine (specific activity, 300 μCi/mm, Amersham/Searle). Certain tubes contained 250 μg of either asialofetuin, asialoagalactofetuin, or β-N-acetylhexosaminidase-treated ovalbumin. Incubations were carried out in 15 ml thick-walled, Corex glass centrifuge tubes for 45 min at 37° in a shaking water bath. Incubations were terminated by the addition of 1 ml cold cacodylate buffer, and the tubes were centrifuged at 15,000 × g for 10 min. Supernatants from tubes containing added glycoprotein acceptors were saved for further processing to determine cell-associated glycosyltransferase activity towards exogenous acceptors while the cell pellets from these tubes were discarded. For processing of these supernatants, 5 ml ice-cold 1% PTA in 0.5 N HCl were added, and the tubes were centrifuged at 15,000 × g for 10 min to collect the precipitate. The precipitate was washed a second time in PTA, dissolved in 0.5 ml NCS, and added to 10 ml Hydromix for liquid scintillation counting. For determination of cell-associated glycosyltransferase activity toward endogenous cell acceptors, the cell pellets from assays without added glycoproteins were saved. The pellets were resuspended in 5 ml 1% PTA in 0.5 N HCl and disrupted by sonic disruption at a probe intensity of 40 for 20 sec. The resultant precipitate was collected by centrifugation, washed a second time with cold PTA, disrupted in NCS, and mixed with Hydromix for scintillation counting. All assays were carried out in triplicate. Parallel control studies contained all reactants except lymphocytes. Cells remained viable during the assay procedure as assessed by trypan blue exclusion exceeding 90% following a 45-min incubation under these conditions. Furthermore, in parallel experiments under identical conditions, cell counts carried out before and after the 45-min assay period demonstrated less than 5% cell loss. This indicates that large-scale cell death with resultant enzyme leakage from dying cells was not a significant factor in this system.

For measurement of glycosyltransferase activities in whole-cell extracts, preparations were made as directed by Gottlieb et al. (8) except that Triton X-100 was used in place of Emulphogene. Briefly, 5 × 10^6 cells suspended in bicarbonate-buffered 0.9% NaCl solution were disrupted by...
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sonic disruption. The sonic product was taken to 0.5% in Triton X-100, incubated 1 hr at 4°, and then centrifuged at 100,000 × g for 60 min to obtain a soluble extract containing glycosyltransferase activities. This material was used to measure β-N-acetylglucosaminyltransferase activity. For extracts enriched for sialyltransferase activity, the original sonically extracted cell suspension was immediately centrifuged at 100,000 × g to remove cell sap. The pellet was taken up in 0.5% Triton in bicarbonate-buffered 0.9% NaCl solution, allowed to stand at 4° for 1 hr to extract the enzyme, and then recentrifuged at 100,000 × g for 60 min. The supernatant was used for assays of sialyltransferase activity. All assays were carried out in 0.01 M Tris-maleate buffer, pH 6.8-0.0125 M MnSO₄ containing radiolabeled nucleotide sugars and exogenous acceptors as directed previously. Reactions carried out at 37° for 45 min were terminated by adding 5 ml cold 1% PTA in 0.5 n HCl, and the mixtures were centrifuged to collect the precipitate, which was washed a second time with PTA, dissolved in NCS, mixed with scintillation cocktail, and counted in a liquid scintillation counter.

RESULTS

Glycosyltransferase Activities of Resting Lymphocytes.
A kinetic analysis of the incorporation of sialic acid, galactose, and N-acetylglucosamine from their respective nucleotide forms into endogenous cellular or added exogenous acceptors by intact resting lymphocytes is presented in Chart 1.

The pattern of glycosyltransferase activity toward endogenous acceptors was similar in every case. Incorporation of radiolabeled carbohydrate into acid-precipitable intracellular material was initiated immediately without a lag period and was more than 90% completed after 20 min. By contrast, incorporation of radiolabeled carbohydrate into exogenous glycoprotein acceptors was characterized by an initial lag period of 10 to 20 min, followed by a period of linear acceleration lasting for 30 to 60 min before activity appeared to plateau. For sialyltransferase activity a plateau level was not reached during the course of the assay.

Specificity of the Assay. The specificity of the assay with intact cells for glycosyltransferase activity towards exogenous acceptors was tested by "crossover" experiments in which each radiolabeled nucleotide sugar was incubated with cells in the presence of "inappropriate" exogenous acceptors. Results are given in Table 1. The data demonstrate no transfer of sialic acid from CMP-[14C]sialic acid to either asialoagalactofetuin or α-N-acetylglucosaminylovalbumin indicating excellent specificity for this enzyme assay. Crossover was apparent in the other assays where some incorporation of radiolabeled sugar into inappropriate acceptors occurred. Nonetheless, nonspecific incorporation of radiolabel remained less than 25% in all cases. In the case of the galactosyltransferase assay, incorporation of [14C]galactose into asialofetuin, an inappropriate acceptor, may reflect removal of some penultimate galactose residues from the fetuin during the acid hydrolysis procedure used to prepare asialofetuin (25). Similarly, incorporation of N-[14C]acetylglucosamine into asialoagalactofetuin may actually indicate that some of the more proximal N-acetylglu-

cosamine residues were released from the fetuin during the chemical removal of sialic acid and galactose residues. By contrast, the transfer of a small amount of [14C]galactose from UDP-galactose to α-N-acetylglucosaminylovalbumin may represent incomplete removal of terminal N-acetylglucosamine units that may then serve as substrates for the addition of galactosyl residues.

If cells were exposed to 4° during the course of the assay...
or if they were first immersed in a boiling water bath, enzyme activity was abolished. These observations suggest that cell viability and active metabolism are essential for the glycosyltransferase activity measured in our system, although we cannot exclude the possibility that these data may reflect the thermal amplitude of the glycosyltransferase system rather than additional cellular metabolic requirements.

Glycosyltransferase Activity in Lymphocytes Transformed with E-PHA or Con A. Table 2 lists the results of experiments with intact lymphocytes to compare the activities of glycosyltransferases in resting lymphocytes and lymphocytes transformed with either E-PHA or Con A. Activities of all 3 enzymes towards both exogenous and endogenous acceptors did not differ between resting and E-PHA-transformed lymphocytes. By contrast, in every case lymphocytes transformed by Con A demonstrated markedly increased activity, ranging from 75 to 600% enhancement of the levels found in resting lymphocytes. The lag phase observed in the kinetic pattern of radiolabel incorporation into exogenous acceptors (Chart 1) was maintained when the Con A-treated cells were analyzed, indicating that the apparent increase in glycosyltransferase activity towards exogenous acceptors given by these cells was not merely an artifact resulting from an enhanced rate of transport across the plasma membrane.

In general, the greater increment in glycosyltransferase activity in the Con A-transformed cells was for activity toward endogenous rather than exogenous acceptors. We believe that this reflects in part the fact that incubation of lymphocytes with Con A may result in the appearance of increased numbers of oligosaccharide units at the cell surface (24). These oligosaccharides may serve as substrates for the activities of the glycosyltransferase enzymes, their presence in increased quantity may serve to increase the pool of endogenous transferase acceptors, and, as a result, the apparent endogenous enzyme activity. Since we have shown previously that Con A- and E-PHA-transformed cells may have different cell surface oligosaccharides (24), it is also possible that the differences in endogenous glycosyltransferase activities observed between them in part may reflect differences in the abilities of the oligosaccharides unique to each group to function as endogenous acceptors. However, the fact that the differences between

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Sugar nucleotide</th>
<th>Glycoprotein acceptor</th>
<th>pmol sugar incorporated/10⁷ lymphocytes/45 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sialyltransferase</td>
<td>CMP-[¹⁴C]sialic acid</td>
<td>Asialofetuin, Asialoagalactofetuin, a-N-Acetylglucosaminylovalbumin</td>
<td>0.45, 0.0, 0</td>
</tr>
<tr>
<td>Galactosyltransferase</td>
<td>UDP-β-[¹⁴C]galactose</td>
<td>Asialoagalactofetuin, Asialofetuin, a-N-Acetylglucosaminylovalbumin</td>
<td>1.24, 0.26, 0.30</td>
</tr>
<tr>
<td>N-Acetylgalactosaminyltransferase</td>
<td>UDP-N-[¹⁴C]acetyl-b-glucosamine</td>
<td>a-N-Acetylglucosaminylovalbumin</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Table 1
Specificity of glycosyltransferase assays
Enzyme assays were carried out with intact cells as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Acceptors</th>
<th>pmol sugar incorporated/10⁷ lymphocytes/45 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sialyltransferase</td>
<td>Endogenous, Asialofetuin</td>
<td>0.18 ± 0.06⁶, 0.20 ± 0.06⁶, 1.08 ± 0.25⁶</td>
</tr>
<tr>
<td>Galactosyltransferase</td>
<td>Endogenous, Asialoagalactofetuin</td>
<td>0.44 ± 0.07⁶, 0.67 ± 0.32⁶, 2.93 ± 0.52⁶</td>
</tr>
<tr>
<td>N-Acetylgalactosaminyltransferase</td>
<td>Endogenous, a-N-Acetylglucosaminylovalbumin</td>
<td>0.49 ± 0.24⁶, 0.78 ± 0.23⁶, 2.64 ± 0.44⁶</td>
</tr>
</tbody>
</table>

Table 2
Glycosyltransferase activities in unstimulated lymphocytes and in lymphocytes stimulated with E-PHA or Con A
Enzyme assays were carried out with intact cells as described in "Materials and Methods." Stimulated lymphocytes were obtained by incubating peripheral blood lymphocytes for 72 hr with either E-PHA, 5 μg/ml, or Con A, 50 μg/ml.

Value significantly different from that of unstimulated lymphocytes (p < 0.05).

Mean ± S.E.
Con A- and E-PHA-transformed lymphocytes were maintained when exogenous acceptors were used indicates that real differences in transferase enzyme activities were present.

Cells incubated for 72 hr in media without mitogen contained levels of glycosyltransferase activities equivalent to those in resting lymphocytes. When Con A, 50 μg/ml, was added directly to the assay systems containing unstimulated lymphocytes, there was no stimulation of glycosyltransferase activities. Although there was no significant loss of cell number or viability during the assay procedure with intact cells, we considered that the glycosyltransferase activities towards exogenous acceptors might result from enzymes released from the cells into the media rather than from cell-associated enzyme activity. For examination of this possibility, resting lymphocytes or Con A-transformed cells were incubated under assay conditions in cacodylate buffer without nucleotide sugars or exogenous acceptors. Supernatants were harvested and then tested for glycosyltransferase activities in our standard assay system from which only the cells were omitted. In each case the amount of radiolabeled sugar incorporated into exogenous acceptors by these cell-free supernatants was less that 10% of the incorporation obtained when cells were present. We conclude that there was no significant release or leakage of glycosyltransferase enzymes from lymphocytes during the assay procedure. In other experiments cell-free supernatants prepared as just described were added to standard assay systems containing cells. Compared to controls tubes containing the supernatants showed no increase in the incorporation of radiolabel into endogenous cellular acceptors. These data serve to minimize the potential role of extracellular lytic enzymes causing sugar nucleotide hydrolysis with resultant increased cellular penetration of free radiolabeled sugar.

It is possible that the glycosyltransferase assay system with intact cells (Table 2) might measure only ectoglycosyltransferase activities located on the cell surface and not accurately reflect the true intracellular activities of these enzymes (17). Therefore, we examined the abilities of Triton X-100 extracts prepared from sonically disrupted lymphocytes to carry out sialyl- and N-acetylglucosaminyltransferase reactions. Results are summarized in Table 3. These data show that relative to resting lymphocytes both transferase activities were stimulated 4- to 5-fold in Con A-transformed cells but remained at baseline levels in cells transformed by E-PHA. Because of differences between the assay systems used to measure activities in intact cells and in cell extracts, the data in Tables 2 and 3 are not directly comparable. However, the relative values are consistent, and both sets demonstrate enhancement of transferase activities in Con A-stimulated cells whereas no effect is seen in E-PHA-stimulated cells.

**Effect of Puromycin on Glycosyltransferase Activity of**

**Table 3**

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Acceptor</th>
<th>pmol sugar incorporated/mg protein/45 min&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Unstimulated</th>
<th>E-PHA</th>
<th>Con A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sialyltransferase</td>
<td>Asialofetuin</td>
<td>2.6</td>
<td>2.9</td>
<td>12.1</td>
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</tr>
<tr>
<td>N-Acetylglucosaminyltransferase</td>
<td>a-N-Acetylglucosaminyl-ovalbumin</td>
<td>78.5</td>
<td>70.7</td>
<td>306.2</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Results represent mean of at least 2 determinations on lymphocytes prepared from different individuals.

**Table 4**

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Acceptor</th>
<th>pmol sugar incorporated/10&lt;sup&gt;6&lt;/sup&gt; lymphocytes/45 min&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Without puromycin</th>
<th>With puromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sialyltransferase</td>
<td>Endogenous Asialofetuin</td>
<td>1.08</td>
<td>0.97</td>
<td>1.43</td>
</tr>
<tr>
<td>Galactosyltransferase</td>
<td>Endogenous Asialoagalactofetuin</td>
<td>2.93</td>
<td>2.07</td>
<td>3.82</td>
</tr>
<tr>
<td>N-Acetylglucosaminyltransferase</td>
<td>Endogenous a-N-Acetylglucosaminyl-ovalbumin</td>
<td>2.64</td>
<td>2.44</td>
<td>3.82</td>
</tr>
</tbody>
</table>

<sup>a</sup> Lymphocytes for the assay were first cultured for 72 hr in the presence of Con A, 50 μg/ml, with or without puromycin, 0.5 μg/ml.
Con A-stimulated Cells. Puromycin, 0.5 μg/ml, was added at the start of 72-hr Con A cultures to evaluate the effect that inhibition of protein synthesis might have on the observed increase of glycosyltransferase activity in Con A-stimulated cells. Table 4 illustrates that puromycin caused only mild inhibition of the expected enhanced levels of transferase activity in the Con A-transformed lymphocytes. Parallel incubations in which [3H]leucine was added at 68 hr to the cells in culture indicated that protein synthesis was inhibited by 93% in these experiments.

DISCUSSION

In a previous communication we reported that incubation of lymphocytes with either Con A or E-PHA resulted in the appearance of new receptors at the cell surface for a group of 6 different plant lectins (24). Although the E-PHA effect could be abolished by the addition of puromycin to the cultures, puromycin had no effect on the appearance of new receptors stimulated by Con A (24). Similarly, while both lectins caused increased incorporation of radiolabeled galactose into cell surface glycoproteins, the glycosylation induced by E-PHA, but not that induced by Con A, was abolished by puromycin (24). We now report that compared to resting lymphocytes Con A-stimulated lymphocytes possessed enhanced capacity to transfer the sugars, sialic acid, galactose, and N-acetylglucosamine from their respective nucleotide donors to both endogenous and exogenous acceptors. The enhanced glycosyltransferase activity induced by Con A was not inhibited in the presence of puromycin despite inhibition of de novo protein synthesis by more than 90%. This indicates that synthesis of new enzymes was not necessary for the observed increases in glycosyltransferase activities. By contrast to the findings in Con A-stimulated cultures, the corresponding glycosyltransferase activities of E-PHA-stimulated lymphocytes did not differ from those of unstimulated cells.

These data suggest that the extensive remodeling of the cell surface saccharide pattern induced by incubation of human lymphocytes with Con A, as previously reported (24), may be due in major part to the glycosylation of pre-synthesized protein units by stimulation of the lymphocyte multiglycosyltransferase system. Conversely, the alteration of cell surface saccharide structure induced by E-PHA requires the synthesis of new protein units and is not associated with enhancement of glycosyltransferase activities. Since the E-PHA-transformed lymphocytes do possess more lectin-binding sites than resting cells (24), the baseline activity of the lymphocyte glycosyltransferase system clearly is sufficient to support at least a modest increase in glycosylation demands.

The mechanism of enhancement of glycosyltransferase activities in Con A-stimulated lymphocytes is unknown. A direct interaction of the lectin with the enzymes is ruled out by our observation that addition of Con A directly to the enzyme assay system containing unstimulated lymphocytes did not enhance activity. Others have reached similar conclusions (10, 31). These data demonstrate a requirement for a period of preincubation of Con A with the lymphocytes, and they suggest that the increased glycosyltransferase activity in Con A-stimulated cells is the end result of a complex series of metabolic events as yet undetermined. Although we have not determined the time course of transferase activation, Painter and White (17) have demonstrated that for mouse thymocytes enhancement of sialyltransferase activity by Con A was complete within 1 hr after addition of the mitogen.

Young et al. (31) studied Con A-induced enhancement of galactosyltransferase activity in purified rat liver Golgi membranes. These workers found as much as 100% enhancement of the activity of the membrane-bound galactosyltransferase by Con A, and they observed that Con A had the effect of decreasing the apparent K_m of the enzyme (31). They postulated that a lectin-induced alteration of the mobility and/or distribution of membrane glycoproteins or increased membrane lipid fluidity might have produced the enhanced activity of the membrane-bound enzyme (31). A similar explanation may underlie our findings with intact lymphocytes. The fact that enhanced transferase activities were also found in the Triton X-100 extracts of Con A-stimulated cells is consistent with this possibility since the native microenvironment of the enzymes may remain intact under the conditions of solubilization used for these studies (9).

Con A has been shown to modify the activities of a number of plasma membrane bound enzymes in various cellular systems. Thus low Con A concentrations increase the Mg^2+-ATPase activity of lymphocyte (15, 18), adipocyte (10), mammary gland (18), and liver (19) cell membranes. In addition plasma membrane 5'-nucleotidase in rat liver cells is inhibited by Con A, apparently through a direct interaction of the lectin with the enzyme (23). Others have described increased glycosyltransferase activities in mouse lymphocytes (6) and thymocytes (12, 17) stimulated by Con A, and a number of workers have provided evidence for a cell surface localization for the multiglycosyltransferase system in lymphocytes (12, 17, 22), but this point is not yet resolved. (5, 11).

Although a small percentage of the total glycosyltransferase activity may be located at the cell surface, the majority of activity is located intracellularly (5, 11, 21), and evidence suggests that most cell surface proteins are glycosylated internally before their insertion into the cell membrane (11, 14). The data in Table 2 with intact cells imply that the glycosyltransferase activities measured in this system may be located at the cell surface. Therefore, it was important to measure enzyme activities in cell extracts (Table 3) to be certain that the activities detected in intact cells were representative of total enzyme activity. Although the data in Tables 2 and 3 are not directly comparable because differences in the assay systems may influence the levels of activities observed, e.g., certain transferase activities may be altered by the presence of detergents (3), results in the 2 sets of experiments were consistent, indicating that measurements of glycosyltransferase activities in intact human lymphocytes may be a valid reflection of the total cellular activities of these enzymes.

A major criticism of glycosyltransferase assays with intact cells has been that hydrolysis of the sugar nucleotide and entry of the free carbohydrate into the cell may account for the findings (11). To minimize this possibility, we have...
measured endogenous incorporation only into acid-precipitable material, thus eliminating from consideration the possible contribution of simple transport of nucleotide sugars or their breakdown products into acid-soluble intracellular pools. Experiments in which cell-free supernatants failed to enhance measured uptake of radiolabel exclude a significant role for substrate hydrolysis in our system. In addition, the fact that our results with intact cells were consistent with those with solubilized cell extracts where uptake into the cells would not be a factor suggests that actual enzyme activity, not sugar transport, was measured. Furthermore, kinetic analysis of the glycosyltransferase-mediated incorporation of radioactivity into endogenous acceptors (Chart 1) indicates that the reaction is initiated immediately without the 10- to 15-min lag phase characteristic of systems in which nucleotide sugars are degraded prior to uptake into the cells (5). Finally, after 20 min of incubation, the increase in rates of radiolabeled sugar incorporation actually decreases and plateaus (Chart 1) despite the fact that less than 0.25% of added nucleotide sugar has been incorporated in that time. If the system were dependent upon uptake of sugars derived from nucleotide sugar breakdown, one would expect incorporation of radiolabel to continue until transport was complete. The possibility that cell damage with release of intracellular enzymes could be the basis for the transferase activities observed towards exogenous acceptors seems ruled out by the observation that there was no significant cell loss during the assay period and that the cells recovered at the end of the assay procedures showed no increase in trypan blue uptake relative to cells tested immediately before. Furthermore, the possibility that release of enzymes from cells during the assay might be responsible for the observed glycosyltransferase activities towards exogenous acceptors was ruled out by the observation that cell supernatants contained no significant glycosyltransferase activities. Although mitogenic lectins have been obtained from a variety of different plants, most of our knowledge concerning the mechanism of activation of lymphocytes by “non-specific” agents such as the plant mitogens, has been gathered with PHA and Con A as group prototypes. Both PHA and Con A appear to specifically activate T-lymphocytes (16, 30) and to induce blastogenesis of 10 to 50% of the initial lymphocyte population present in culture (16). Kinetically, the mitogenic responses to both lectins are similar (16, 30), and Con A- and E-PHA-transformed lymphoblasts appear morphologically identical by light microscopy. For these reasons it is often assumed that the plant mitogens all have similar if not identical modes of action, and further, that experimental observations valid for one mitogen will be valid for others as well. The fact that E-PHA and Con A have different effects on the organization of complex carbohydrates at the lymphocyte membrane (24) and the fact that these changes may be mediated by different effects on both the protein synthetic apparatus (24) and on the multiglycosyltransferase enzyme system strongly imply that such assumptions are not warranted. These studies provide strong evidence that all lectin-transformed lymphocytes are not the same, and they further suggest the possibility that functional differences may be associated with the observed biochemical and metabolic differences among lymphocytes transformed by different lectins.

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