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Expression of UDP-*N*-acetylgalactosamine:β-galactose β1,4-*N*-acetylgalactosaminyltransferase in functionally defined T-cell clones

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To measure UDP-*N*-acetylgalactosamine:β-galactose β1,4-*N*-acetylgalactosaminyltransferase (β1,4-GalNActransferase) in crude cell and tissue extracts we designed an assay containing UDP-[^{3}H]*N*-acetylgalactosamine as donor and biotinylated human glycophorin A as an acceptor. After incubation the labelled acceptor was separated by the use of avidin–agarose from extract-derived endogenous acceptors. This assay permitted one to measure specifically the β1,4-GalNActransferase in crude extracts. This glycosyltransferase has previously been shown to be involved in the biosynthesis of *Vicia villosa* (hairy winter vetch)-lectin (VV)-binding sites of the murine cytotoxic T-cell line B6.1. Since VV-binding sites are a distinct marker for the cytotoxic subclass of murine T-lymphocytes, we used this assay to determine enzyme levels in a panel of functionally defined murine T-cell clones. Non-cytolytic T-cell lines generally have low activity, whereas most cytotoxic lines have high levels of activity. However, one cytotoxic T-cell line does not express the enzyme, although it has large numbers of VV-binding sites. This suggests the existence of another type of VV-binding sites which is independent of the β1,4-GalNActransferase in some cytotoxic-T-lymphocyte lines. The enzyme was also assayed in a variety of other tissues and found to have a very high activity in the intestine but a low activity in most other tissues. This was in considerable contrast with the ubiquitously high expression of UDP-GalNAc:peptide α1-GalNActransferase. Therefore the β1,4-GalNActransferase seems to be regulated during differentiation.

INTRODUCTION

The use of lectins as probes for surface oligosaccharide structures has revealed a surprising heterogeneity among lyphocytes [1], and some lectins define subpopulations with distinct functional properties. For instance, in mice, peanut (*Arachis hypogaea*)-lectin binding has been found to be restricted to functionally immature cells within the thymus [2] and to be induced by antigen on germinal-centre B-lymphocytes [3–5]. Similarly, *Vicia villosa* (hairy winter vetch) lectin (VV) has been demonstrated to bind to a subset of mixed leucocyte culture (MLC) cells to extents varying between 20 and 60% [6,7,8]. Moreover, VV seems to bind preferentially to cytotoxic T-lymphocytes (CTLs), this being based on the following findings. (1) The 30–50% of cells of an MLC population that can be retained on a VV–Sepharose column and can be eluted by *N*-acetylgalactosamine (GalNAc) contain all of the cytolytic activity, with no cytolytic activity present in the run-through fraction [6]. (2) MLC populations (which contain cytotoxic cells) bind 16-fold more VV than do thymocyte or spleen-cell populations [6]. (3) Cloned long-term CTL lines bind 100–1000-fold more VV than do various T-cell lymphoma lines [9]. (4) Recently, a dramatic increase of VV binding has been demonstrated to occur in MLC populations repeatedly re-stimulated with antigen plus recombinant interleukin 2 (IL-2) [10] and also during the induction of cytolytic activity by lymphokines in a T-cell hybrid [11]. (5) The expression of VV-binding sites is correlated with cytolytic activity in a series of T-cell hybrids between CTL lines and an AKR-mouse thymoma [12]. (6) The binding of VV to CTL generated in primary MLC is likely to be mediated by T 145, a 145 kDa surface glycoprotein that is the main surface glycoprotein capable of binding to VV–Sepharose and which is expressed by MLC blasts generated across MHC (major histocompatibility locus)-Class-I-antigen incompatibilities but not by blasts from Mls-antigen-incompatible or MHC-Class-II-incompatible MLCs or by unstimulated T- and B-lymphocytes [6,13]. In contrast, long-term CTL lines express many more glycoproteins which can bind to VV–Sepharose [9].

However, the correlation of VV-binding sites with cytolytic activity is not absolute, inasmuch as T 145-negative CTL can be generated *in vivo* [14]. Also, some workers have failed to separate CTLs from other cells in primary MLCs on the basis of VV binding [15]. Unfortunately, most studies used a mixture of two isolectins present in VV extracts that have different specificities [16,17] (see Table 3 below).

Biochemical analysis of the VV-binding sites of a cloned CTL line, B6.1, showed that it possesses GalNAc residues in β1,4 linkage to galactose on *O*-linked oligosaccharides [18]. An analogous structure has previously only been described in glycophorin from

Abbreviations used: Con A, concanavalin A; CTL, cytotoxic T-lymphocyte; CS, interleukin-2-containing conditioned medium from concanavalin A-stimulated rat spleen cells [30]; FCS, fetal-calf serum; FITC, fluorescein isothiocyanate; GalNAc, *N*-acetylgalactosamine; IL-2, interleukin 2; MLC, mixed leucocyte culture; MHC, major histocompatibility locus; PBS, phosphate-buffered saline (for composition, see the text); PMA, phorbol myristate acetate; VV, *Vicia villosa* (hairy winter vetch) lectin; β1,4-GalNActransferase, UDP-*N*-acetylgalactosamine:β-galactose β1,4-*N*-acetylgalactosaminyltransferase; PAGE, polyacrylamide-gel electrophoresis.

individuals having the rare human blood-group type Cad [19] (see Table 3 below). This β1,4-linked GalNAc residue is necessary for VV binding by this line because a VV-resistant mutant of B6.1, VV6, with 100-fold lower binding capacity, lacks β1,4-linked GalNAc residues and also lacks the corresponding β1,4-GalNActransferase [9,18,20].

In the present study we investigated (i) whether CTL lines other than the previously reported B6.1 and BD.2 lines contain the β1,4-GalNActransferase, (ii) whether short-term generation of CTL by Con A is associated with an induction of this enzyme and (iii) whether tissues other than lymphocytes contain this β1,4-GalNActransferase.

EXPERIMENTAL

Mice

All mice were obtained originally from Jackson Laboratories and bred at our colony in Epalinges.

Cells

The origin and characteristics of cell lines are described in Table 1 and Fig. 4 (below). In general, cells were grown in Dulbecco's modified Eagle's medium with 5–10% (v/v) FCS and appropriate growth factors and stimulator cells if required. For enzyme assays, the cells were collected, stimulators were removed by density separation in Ficoll–Hypaque (Seromed, München, Germany), cells were washed three times by centrifugation at 250 *g* for 10 min in 10 ml of PBS, counted and frozen as a pellet at −20 °C. Care was taken to remove as much PBS as possible before freezing the cell pellet. The cytolytic activity of all clones was high at the time of testing.

Chemicals

UDP-[1-^{3}H]GalNAc (11 Ci/mmol) and Aquasol were from New England Nuclear Corp. NaB^3H_4 (15 Ci/mmol) was from Amersham International. Aprotinin (A 1153), *N*-hydroxysuccinimidobiotin (H 1759), avidin–agarose [A 5150; capacity 20 μg of D(+)-biotin/ml] and VV–FITC (L 5887) were from Sigma. Con A was from Pharmacia and Tritons X-100 and X-114 were from Serva. Pronase was from Calbiochem. Apo-mucin was a gift from Dr. A. Elhammer and prepared as described by Elhammer & Kornfeld [21].

Buffers

PBS was 150 mM-NaCl/10 mM-PO_4^{3-}, pH 7.4. Buffer A was 1% Nonidet P40/1 M-NaCl/10 mM-Tris/HCl, pH 7.5. Buffer B was 0.5% (w/v) Triton X-100/10 mM-Tris/HCl (pH 7.0)/2 mM-$MgCl_2$. Buffer C was 100 mM-Tris/HCl (pH 8)/20 mM-$CaCl_2$. Buffer D was 1% (w/v) Triton X-114/10 mM-Tris/HCl (pH 7.0)/75 mM-NaCl/aprotinin (1 μg/ml). Buffer E was 10 mM-Tris/HCl (pH 7.0)/2 mM-$MgCl_2$.

Preparation of glycophorin–biotin

Glycophorin was partially purified as described previously [20] and either biotinylated directly or further purified on a column (100 cm × 1.5 cm) of Sephadex

Table 1. Phenotypes of cloned T-lymphocyte lines

Name	Haplotype	Function	Specificity	Lyt-2*	L3T4*	Feeder-cell-dependence	Ref.
B6.1	b	CTL	H-Y,H-2D^d	+	−	No	[27]
14-7	d/b	CTL	Influenza virus	+	n.t.†	Yes	[28]
14-7fd	d.b	CTL	Influenza virus	n.t.	n.t.	No	[28]
Clone 11	b	CTL	H-2^d	+	−	Yes	[29]
Clone 14	b	CTL	MSV,H-2^b	+	−	Yes	[29]
Clone 16	b	CTL	H-Y	+	−	Yes	[29]
B1.8.11	n.t.	CTL	H-2^b	+	n.t.	Yes	‡
DFL-1	d	CTL	Fluorescein	n.t.	n.t.	No	[30]
DFL-2	d	CTL	Fluorescein, D^d	n.t.	n.t.	No	[30]
CFL-1	d	CTL	Fluorescein	n.t.	n.t.	No	[30]
Clone 3	bm^{12}	IL-2 production	Ia^b	−	+	Yes	§
Clone B3	d	IL-2 production	Mls^a	−	n.t.	Yes	[31]
B2-16	b	IL-2 production	Horse apo-cytochrome *c*	−	+	Yes	[32]
C3	b	MAF‖-producing	PPD¶	−	+	Yes	[38]
C10	b	MAF-producing	PPD	−	+	Yes	[38]
D18	b	MAF-producing	PPD	−	+	Yes	[38]

* + and − indicate the presence or absence of surface markers.
† n.t., not tested.
‡ CR × CD anti-C57BL/6 CTL clone (M. Nabholz, unpublished work).
§ C57BL/6 bm 12 anti-C57BL/6 T-cell clone produced by H. R. McDonald (unpublished work).
‖ MAF, macrophage activating factor.
¶ Purified protein derivative of *Mycobacterium tuberculosis*.

G-100 in water. The resulting preparation was free of contaminants as judged by SDS/PAGE followed by silver staining. Biotinylation was done using either a 5:1 or 10:1 molar ratio of D-biotin succinimide ester to glycophorin monomer at 1 mg of protein/ml for 2 h at room temperature. Free biotin succinimide was removed by dialysis. The 'best' reagent obtained contained 3.2 biotin molecules per glycophorin monomer.

Preparation of tissue extracts

We used a modification of the procedure of Bordier [22] to purify membrane proteins. Organs were weighed and homogenized in a 10-fold amount of cold buffer D in Potter–Elvehjem homogenizer with the Teflon pestle rotating at 600 rev./min for 60 s (~ 20 strokes). The extracts were left on ice for 30 min and centrifuged at 10000 *g* for 10 min at 4 °C. The pellet was discarded. The supernatant was warmed to 30 °C for 3 min, centrifuged for 30 s at 10000 *g* and the supernatant was discarded. The precipitation was repeated twice by adding 1 ml of ice-cold buffer E. The final detergent pellet was diluted with 3 vol. of buffer B to be used in standard assays. Triton X-114 extraction was also used for the B6.1 line when used as a positive control in a tissue-distribution experiment.

Assay for β1,4-GalNActransferase

Cell pellets were thawed and extracted at $(0.6–1.2) \times 10^5$ cells/μl in buffer B for 60 min on ice; lysates were centrifuged at 10000 *g* for 10 min at 4 °C and supernatants used for assays. Standard assay mixtures for cell lines contained 50 mM-Tris/HCl, pH 7.5, 10 mM-$MnCl_2$, 50 nM-2-mercaptoethanol (added to the $MnCl_2$ stock solution to prevent oxidation), 0.2 μCi of [1-^{3}H]GalNAc or UDP-[6-^{3}H]GalNAc, 50 μM-UDP-GalNAc, 10 μg of biotin–glycophorin, 0.25 mg of Triton X-100 and variable amounts of cell extracts [usually 10 μl; equivalent to $(0.6–1.2) \times 10^6$ cells]. For Triton X-100 extracts this corresponds to an average of 90 ± 40 μg of protein per assay (range 30–170 μg). The final volume was 50 μl. Control tubes contained buffer B instead of enzyme or water instead of glycophorin–biotin.

Reactions were started by adding cell extract and incubating for 30 min at 37 °C with occasional shaking. The reaction was stopped by placing tubes on ice. Tubes received 150 μl of buffer B, were vortex-mixed and centrifuged for 2 min at 10000 *g* to remove precipitates that sometimes formed during the incubation. Control experiments showed that the glycophorin–biotin stayed in the supernatant in this step. A 170 μl portion of the supernatant was transferred to fresh tubes containing 1 unit of avidin–agarose and incubated for 20 min at 4 °C with frequent shaking. The beads were then washed six times in 1 ml portions of buffer A and incubated with Pronase (2 μg/sample) at 56 °C in buffer C for 2 h. Beads and buffer were then transferred to plastic vials, mixed with the liquid-scintillation cocktail Aquasol and counted for radioactivity. The mixture for assays of tissue extract contained AMP (5 mM) to prevent breakdown of substrate.

In each cell or tissue extract we checked for inhibitors by assaying test extract mixed with extract from B6.1. The percentage inhibition was calculated by the formula:

$$1 - \frac{\text{Incorporation in mixture}}{\text{Sum of incorporations of test lysate and B6.1 assayed separately}} \times 100$$

Other methods

UDP-GalNAc:peptide α-1-*N*-acetylgalactosaminyltransferase was assayed as described previously [21]. Protein content of detergent extracts of cells or tissues was determined by the method of Lowry *et al.* [23] with bovine serum albumin as standard. [6-^{3}H]GalNAc was prepared as described by Nelsestuen & Kirkwood [24], giving a final specific radioactivity of 600 mCi/mmol.

RESULTS

Reduction of endogenous background incorporation in β1,4-GalNActransferase assays

When cell lysates from the previously used CTL-line B6.1 are incubated with UDP-[^{3}H]GalNAc and glycophorin as an acceptor followed by precipitation with trichloroacetic acid, the incorporation of radiolabel into endogenous acceptors can amount to as much as 50%, thereby limiting the sensitivity of the assay [20]. In order to separate the endogenous acceptors from the glycophorin, we 'tagged' the latter with biotin and isolated it after the incubation with the cell lysate by means of avidin–agarose (see the Experimental section). This modification reduced the incorporation of [^{3}H]GalNAc in the absence of exogenous acceptors to low levels (Fig. 1*a*) and allowed the measurement of enzyme activity over a large range of enzyme concentrations (Fig. 1*c*) in a specific and sensitive way. Nevertheless, glycosyltransferase assays in crude tissue or cell extracts are subject to errors attributable to the possible presence of enzymes which degrade the glycosyltransferase, the acceptor, the substrate or the newly formed product, or to the presence of inhibitors of the transferase. To control for these possibilities we routinely performed mixing experiments where B6.1 lysate was assayed together with lysate from test samples.

Presence of β1,4-GalNActransferase in functionally characterized cloned T-cell lines

Fig. 2 shows the incorporation of [^{3}H]GalNAc into glycophorin obtained in nine independent CTL lines and six helper-cell lines, the characteristics of which are summarized in Table 1. In all assays we performed mixing experiments with B6.1 lysate and found inhibitions from 0 to 20%, indicating that the absence of activity is not due to inhibitors in any cell lines. Together with the results from the BD.2 CTL line published previously [20], we found that six out of ten independent CTL lines have relatively large amounts of enzyme (500 c.p.m./100 μg), three have intermediate amounts (150–500 c.p.m./100 μg) and one line (C114) is completely deficient. On the other hand, two out of six helper-cell clones have intermediate amounts of β1,4-GalNActransferase and four lines have low levels (0–150 c.p.m./100 μg). Surprisingly, the CTL line clone 14 in which β1,4-GalNActransferase could not be detected had as many binding sites for VV as B6.1 (Fig. 3).

Fig. 4 summarizes the results obtained with various non-cytolytic leukaemic cell lines. With the exception of a subline of Yac, all of them are negative. Some of these lines have previously been found to lack VV-binding sites, but Yac had not been tested. The EL-4.Ou.Br.6.1 line, which can be induced by PMA to secrete high amounts of IL-2 and therefore resembles a helper line,

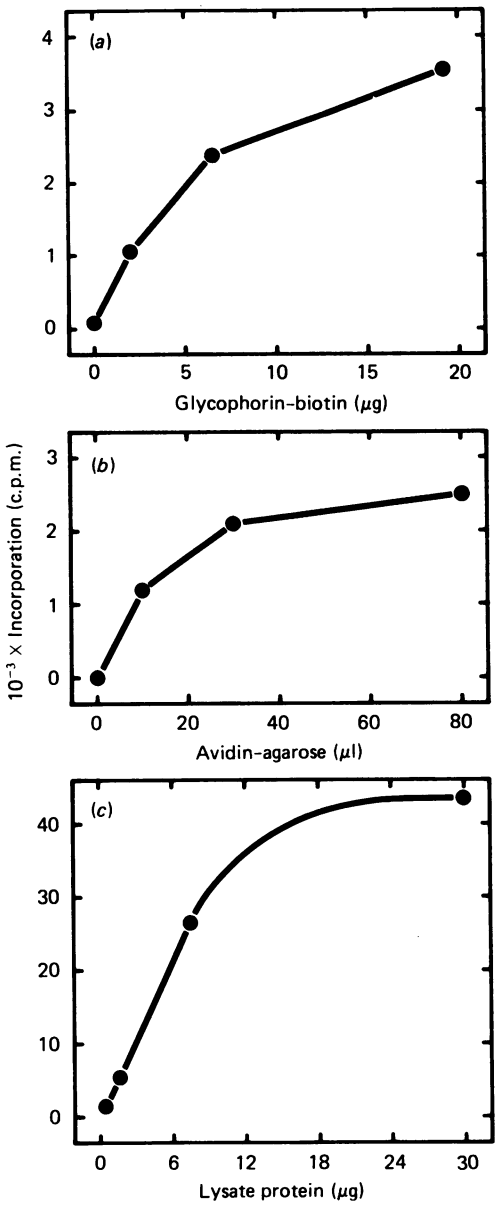

Fig. 1. Titration of glycophorin-biotin, avidin–agarose and cell extract

For (*a*) and (*b*) 15 μl of a Triton X-100 extract of B6.1 was used in a standard enzyme assay. In (*a*) the amount of glycophorin–biotin was varied and followed by 80 μl of avidin–agarose. In (*b*) a fixed amount of glycophorin–biotin (6.4 μg) was followed by various amounts of avidin–agarose. In (*c*), a Triton X-114 extract from colon was assayed in a standard assay (10 μg of glycophorin, 60 μl of avidin–agarose) using various amounts of lysate.

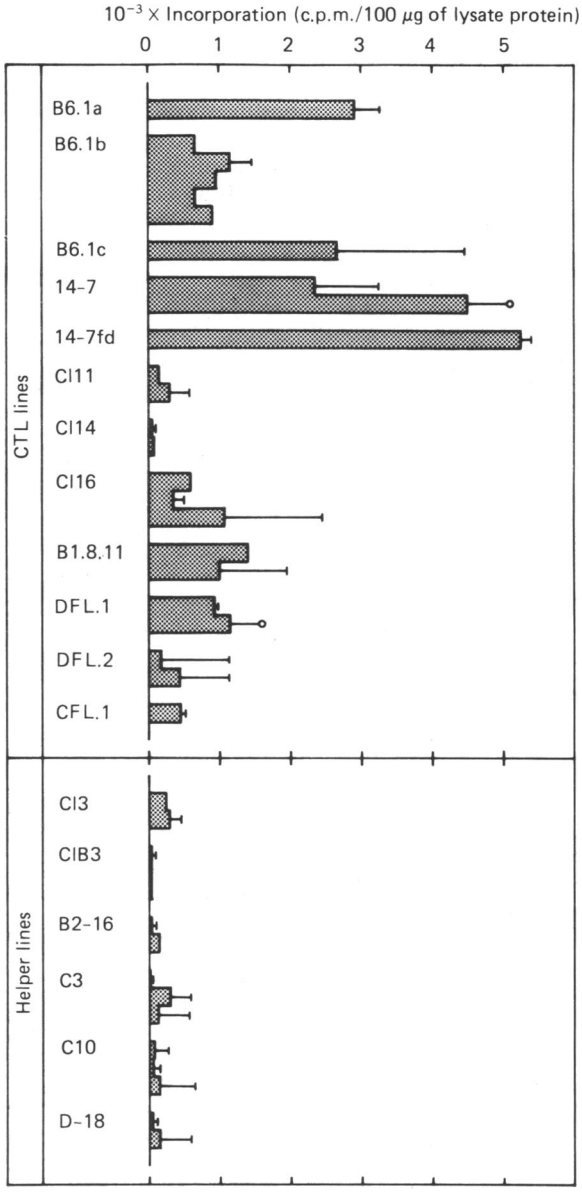

Fig. 2. β1,4-GalNActransferase in various cloned cytotoxic or helper T-lymphocyte lines

Triton X-100 extracts of cell lines were assayed under standard conditions in ten different experiments carried out over a period of more than 1 year under approximately identical conditions, although different batches of glycophorin–biotin and other reagents were used. Each bar represents the result for an individual assay in which controls without glycophorin and mixing with B6.1 extracts were done in parallel. Results are expressed as c.p.m. incorporated/μg of lysate protein, and ±S.D. values for duplicate assays, where performed, are indicated on a non-expanded (—o) or an 10-fold-expanded (—|) scale. Incorporation in the absence of glycophorin was consistently low and ranged, for B6.1, from 2.1 to 4.6% of incorporation measured in the presence of glycophorin–biotin. For B6.1, three different cultures (a,b,c) were used.

does not express the enzyme either in the uninduced or in the induced state.

Somatic-cell hybrids between CTL lines and the mouse T-lymphoma BW5147 have been found to be either VV-sensitive, IL-2-dependent and cytotoxic or VV-resistant, IL-2-independent and non-cytotoxic [12,25]. The latter type was found to arise as a rare variant from the former. Two such IL-2-dependent hybrids, MN12.19.4 and T2.16.8.15, have been assayed and contain intermediate and high amounts of enzyme respectively, whereas their IL-2-independent variants are completely deficient. This finding again confirms and extends our previous conclusion [20], namely that the VV-binding capacity of at least some cloned long-term

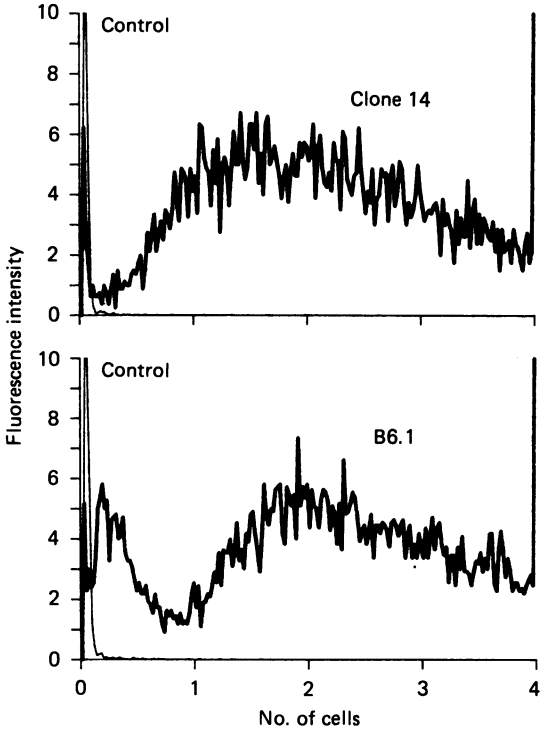

Fig. 3. Fluorescence flow cytometry of cells treated with VV–FITC

B6.1 and clone 14 cells were stained in the cold and in the presence of 0.02% NaN_3 to VV–FITC at 33 μg/ml. GalNAc was added at 10 mg/ml to controls in addition to VV–FITC. After removal of unbound VV–FITC, the fluorescence of individual cells was determined by fluorescence flow cytometry. The number of cells and fluorescence intensity are given in arbitrary units. The two cell lines gave overlapping forward light-scattering profiles (not shown).

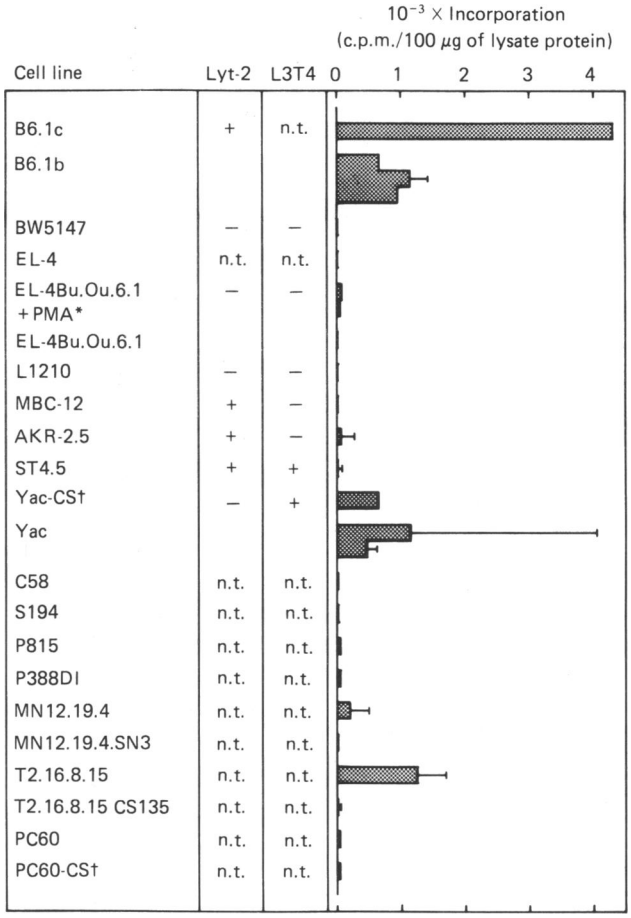

Fig. 4. β1,4-GalNActransferase in various cell lines

Bars represent the incorporation of [³H]GalNAc into glycophorin by Triton X-100 lysates of different cell lines grown for 3 days in the presence of CS in a standard assay. S.D. values for duplicate assays are given at a 10-fold-expanded scale (—|). Inhibition of the activity of B6.1 determined in mixing experiments varied between 0 and 40% and could therefore not account for the general absence of activity in most cell lines. BW5147, EL-4, PMA. C58 is a rat T-lymphoma, S194 is a mouse T-lymphomas, EL-4Bu.Ou.6.1 is a subline of EL-4 originally provided by Dr. J. J. Farrar (National Institutes of Health, Bethesda, MD, U.S.A.), subcloned by Dr. R. MacDonald, which produces IL-2 on stimulation by PMA. C58 is a rat T-lymphoma, S194 is a mouse myeloma, P815 a mouse mastocytoma and P388D1 a mouse macrophage-like line. MN12.19.4 and T2.16.8.15 are IL-2-dependent hybrids between BW5147 and CTL lines, MN12.19.4.SN3 and T2.16.8.15. CS135 are IL-2-independent variants of these lines [12,25]. PC60 is a hybrid between B6.1 and C58 [26]. Surface expression of Lyt-2 and L3T4 are indicated as far as known. Two different thawings of B6.1 (b, c) were used. Notes: *10^6 cells with 10 ng of PMA/ml or medium cultured for 40 h; †cells grown without IL-2-containing conditioned medium. '+', '−' and 'n.t.' are explained in Table 1.

CTL lines is due to the expression of the β1,4-GalNActransferase.

In PC60, a hybrid between B6.1 and the rat T-lymphoma C58, which grows independently of IL-2 but in which IL-2 in conjunction with IL-1 induce cytotoxic activity [26], we could not detect β1,4-GalNActransferase either before or after induction (Fig. 4).

No activity could be found in unstimulated spleen lymphocytes. Moreover, repeated attempts to detect activity in blasts generated in MLC stimulation with Con A or lipopolysaccharide did not induce detectable levels of activity (Fig. 5).

Tissue distribution of β1,4-GalNActransferase

In view of the restricted occurrence of the β1,4-GalNActransferase in the lymphoid system, we investigated whether the enzyme is detectable in other tissues as well. Triton X-100 extracts of most tissues, with the exception of brain, strongly inhibited the β1,4-GalNActransferase activity of B6.1. Therefore we switched to the use of the detergent Triton X-114, which allows the separation of membrane proteins from the bulk of soluble proteins [22]. The β1,4-GalNActransferase activity of B6.1 was recovered in excellent yield in the membrane-protein fraction. However, this protocol could not completely remove the inhibitor(s) found in most of the tissues. The nature of these inhibitors is not clear at present. The specific enzyme activity in Triton X-114 extracts is at least an order of magnitude higher than in Triton X-100 extracts. The small and large intestine and the gall-bladder contain very high β1,4-GalNActransferase activities, whereas in other tissues activities are low. Tissues in which inhibitory

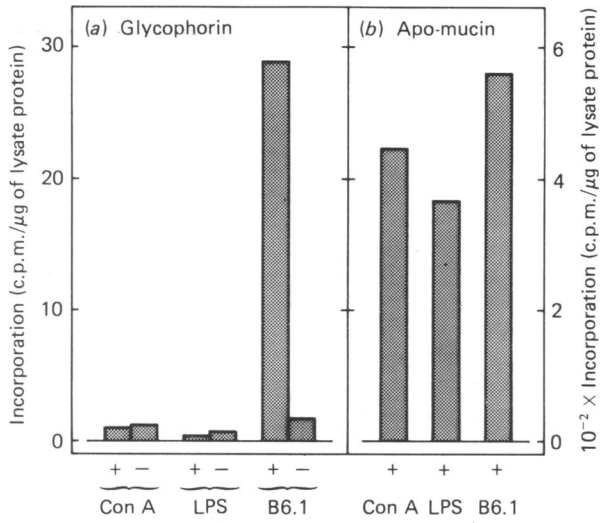

Fig. 5. Enzyme activity in Con A blasts and lipopolysaccharide (LPS) blasts

Spleen lymphocytes from female CBA/J mice were activated *in vitro* either with Con A (2 μg/ml) or lipopolysaccharide (50 μg/ml) for 4 days. Lymphoblasts were washed, incubated in PBS containing 20 mM-α-methyl mannoside and washed several times before lysis. Triton X-100 lysates were assayed with (+) or without (−) glycophorin–biotin (*a*) or apo-mucin (*b*) for 3 and 10 min respectively. Assay mixtures contained between 33 and 66 μg of cellular protein and results are normalized with regard to cellular protein and assay time.

activity was low are listed in Table 2. By contrast, the UDP-GalNAc:serine/threonine α1-GalNActransferase is expressed at similar levels in all tissues tested. Mixing extracts of B6.1 and colon extracts indicates that B6.1, which has been used as a positive control throughout the present study, does not contain any inhibitors for β1,4-GalNActransferase.

DISCUSSION

The use of biotinylated glycophorin has significantly increased the sensitivity and specificity of the β1,4-GalNActransferase assay with regard to our previously used procedure [20] in which the incorporation of [^{3}H]-GalNAc into glycophorin was determined by precipitating, with trichloroacetic acid, the reaction mixtures after incubation. This is probably mainly due to the elimination of reaction products of the UDP-GalNAc:peptide α1-GalNActransferase, which is expressed at a high level in all tissues (Table 2) and which probably accounts for most of [^{3}H]GalNAc incorporation into endogenous trichloroacetic acid-precipitable acceptors of the cell lysate. The modified assay allowed us to measure this enzyme in extracts from various functionally defined T-cell lines and several tissues.

The study clearly shows that the β1,4-GalNActransferase is expressed at enormously variable levels. This is unlike the case for most glycosyltransferases which make the core structures of *N*- and *O*-linked oligosaccharides and which are expressed more or less equally in all tissues (the UDP-GalNAc:peptide α1-GalNActransferase measured in the present study belongs to this group). In contrast, several glycosyltransferases which add the

Table 2. β1,4-GalNActransferase in various tissues

The detergent phase containing membrane-associated proteins of Triton X-114 extracts from various tissues from adult female Balb/c mice and B6.1 were assayed for GalNActransferases by using UDP-[^{3}H]GalNAc as substrate under standard conditions. Results of four independent assays are summarized and expressed as c.p.m. incorporated/30 min per μg of membrane protein using either glycophorin–biotin or apo-mucin as acceptors. For the latter acceptor, the incorporation of [^{3}H]GalNAc in the absence of exogenous acceptor was also determined and was subtracted (mean 14%; range 3.3–35% of incorporation with apo-mucin). Incorporation into glycophorin represents mean values for duplicate or triplicate assays; the other values are single determinations. Inhibitors of the transfer to glycophorin were checked for by mixing with either B6.1 or colon extracts.

	Incorporation (c.p.m./μg)			Inhibition (%) of:	
Tissue	None	Glycophorin	Apo-mucin	B6.1	Colon
Spleen*		3	2730	96	
Lung		0			52
Salivary gland		5		18	
Stomach†		17	2390		0
Small intestine†		467	1792	37	
Caecum	17	3606	2082		
Colon		772	2964	0	
	10	2153			
		945			
	18	3433			
Gall-bladder		140		0	
B6.1		148	334		
		208			
		79			8

* Lymphocytes were removed by pressing the organs on to a stainless-steel screen and tissue retained by the screen was used for assay.

† Bowels were cut open and freed of content by rinsing extensively under cold running tap water. The epithelial layer was then scraped off with a scalpel and lysed in Triton X-114.

terminal or outer sugars such as fucose [33], polylactosamines [33,34], or the brain-specific sialyltransferases which produce polysialosyl units [35] are probably expressed in only a few organs and have stimulated speculations about their involvement in tissue-specific functions. The β1,4-GalNActransferase clearly belongs to this latter group. In many tissues the enzyme is undetectable, although the presence of inhibitors obscures the result. However, since the inhibition in many cases is incomplete (60–90%), we assume that the enzyme levels are low in tissues such as lymph-node, thyroid, testis, ovary and kidney. In contrast, the intestinal epithelia contain in the order of 10–20-fold more enzyme than the B6.1 clone, although the amounts present in B6.1 are already sufficient to transfer GalNAc to most of the *O*-linked oligosaccharides [18]. Further studies will be required to determine whether the high levels of enzyme activity in the intestine are contained in epithelial cells where they might be used to synthesize secretory

Table 3. Structure of *O*-linked oligosaccharides implicated in binding to VV [16,17,19]

Structure	Designation	Binding to VV
GalNAcα1-Ser/Thr	Tn	B_4
GalNAcβ1-4Galβ1-3GalNAcα1-Ser/Thr (α2-3 NeuAc; α2-6 NeuAc)	Glycophorin of Cad$^+$	B_4
GalNAcα1-3Gal-GlcNAc- (α1-2 Fuc)	Blood group A	A_4

products such as mucins or are localized at the plasma membrane as seems to be the case for the UDP-galactose:GlcNAc β1,4-galactosyltransferase in the human intestine [36] or if the enzyme is localized in other components of the mucosa or even in intra-epithelial lymphocytes.

The main purpose of the present study was to see if the association of cytolytic activity with the expression of VV-binding sites can be explained by a CTL-specific expression of the β1,4-GalNActransferase. The correlation has been documented only for blast cells obtained from a single antigenic stimulation *in vitro*. We cannot detect enzyme activity in CTL of this kind (Con A or MLC blasts). However, this might be expected, since Con A blasts contain 100-fold less VV-binding sites on a per cell basis than do B6.1 [9]. Therefore the VV-binding sites of Con A blasts might well be synthesized by the β1,4-GalNActransferase, but the enzyme activity is below the level of detection.

Long-term CTL clones have been found to express very large numbers of VV-binding sites and to contain a multitude of VV-binding surface proteins [9], but comparable data for helper-cell clones are not available. A single helper-cell clone was recently analysed and found to contain much lower levels of VV-binding sites than a CTL clone [10]. We find high levels of β1,4-GalNActransferase only in cytotoxic clones (six out of ten), whereas helper-cell clones have low or intermediate levels of the enzyme.

Much to our surprise, we found one CTL clone, clone 14, which completely lacked β1,4-GalNActransferase, but still expressed the same number of VV-binding sites as did B6.1. Measurements of β1,4-GalNActransferase and VV-binding sites in clone 14 were made at days when the cell line had high cytolytic activity and therefore temporal fluctuations in cytotoxic activity do not seem to play a role. Also, mixing of B6.1 cells with clone 14 before lysis in detergent did not reveal an unstable inhibitor of β1,4-GalNActransferase for the biosynthesis of its VV-binding sites. Unless enzyme levels that are below the detection limit of our assay are sufficient to synthesize a high number of VV-binding sites, it would therefore seem likely that some CTL lines such as clone 14 have another type of VV-binding site which is not made by the β1,4-GalNActransferase. Such structures might be the ones typified by Tn or A blood-group antigens (Table 3) or some other novel structure.

Recently an antibody detecting a carbohydrate determinant expressed strongly on all CTL lines tested and only weakly in helper-cell lines and primary MLC populations has been described [37,10,11]. Since this marker is also correlated with the expression of VV-binding sites, it might be related to the structures synthesized by the β1,4-GalNActransferase [10].

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