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Structural characterization of a low molecular weight receptor for peanut agglutinin in murine lymphocytes

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ABSTRACT

A novel low molecular weight (**LMW**) receptor for the lectin peanut agglutinin (**PNA**) was characterized and purified from murine thymocytes and peripheral lymphocytes in the spleen. Preliminary binding assays with the fluoresceinated lectin demonstrated that the majority of cortical thymocytes, and only a fraction of lymphocytes in the splenic white pulp, as well as other peripheral lymphoid organs were PNA-positive. This positivity was selectively inhibited by the preferred PNA disaccharide ligand (Galβ1,3GalNAc), indicating the specificity of the binding. PNA receptors were purified from thymocytes and splenocytes by affinity chromatography on a PNA-agarose column, and their structural characteristics assessed by treatments with endoglycosidases and alkaline borohydride and analysis by two-dimensional (2-D) gels. Comparisons based on 2-D gels of glycosylated and deglycosylated forms were consistent with the thymic and splenic receptors, sharing a common 21 kDa polypeptide backbone, which is subjected to differential post-translational N-linked glycosylations with one (thymic PNA receptor) or two and/or three (splenic PNA receptor) complex-type glycan units of distinct structures. Analyses of amino acid and carbohydrate compositions of the intact receptors confirmed these observations and revealed a comparatively high level of sialic acid residues in the splenic **PNA** receptor.

INTRODUCTION

Classic studies with the lectin PNA have demonstrated that during the course of intra-thymic development, the pattern of expression of thymocyte cell-surface carbohydrates changes (Reisner *et al.* 1976). PNA, which expresses a high binding preference to the disaccharide sequence Galβ1,3GalNAc (Sharon 1983), selectively recognizes immature cortical thymocytes and not mature medullary cells (London *et al.* 1981; Lefrancois *et al.* 1985; Pulido & Sanchez-Madrid 1990). During thymic maturation, the conversion of the PNA^+ to the PNA^- lymphocyte-phenotype has been attributed to increased levels of sialyltransferase activity and the subsequent masking of the PNA disaccharide ligand with terminal sialic acid (Dall'Olio *et al.* 1991; Gillespie *et al.* 1993). Although the functional consequences of this glycosylation change is not, as yet, elucidated, it has been postulated that the extent of the

PNA receptor sialylation may have implications in modulating cell-cell interactions during thymocyte maturation, selection or migration (DeMaio *et al.* 1986; Schulof *et al.* 1987).

Understanding the precise functional significance of PNA receptors prompts detailed structural analyses of these glycoproteins, as well as the number and type of their associated oligosaccharides, in different cell types. On thymocytes, the majority of the PNA binding sites appear to be carried on two cell-surface glycoproteins; the 115 kDa leukosialin (CD43) and the leukocyte common antigen (CD45), expressed as a molecular complex of several isoforms ranging from 180 to 220 kDa, both are known to be extensively O-linked glycosylated (Brown & Williams 1982; Cook *et al.* 1987; Pulido & Sanchez-Madrid 1990). PNA-binding glycoproteins of variable sizes have also been detected on several malignant cell lines (Miyauchi *et al.* 1982; Flavell *et al.* 1989; Goulut-Chassaing *et al.* 1992), although there is no available information on either their structural characteristics in peripheral lymphocytes or their putative modulations that may accompany thymocyte maturation.

In the present study, we report on the purification and characterization of a novel low molecular weight PNA-binding glycoprotein derived from murine thymocytes and splenocytes. In both cell-types evidence is presented that, a common 21 kDa polypeptide back-bone is subjected to differential post-translational additions of PNA disaccharide ligand-bearing complex N-linked glycans, which seem to account solely for the structural heterogeneity of the glycosylated receptor in the different lymphoid organs. The significance of these findings is discussed in relation to mechanisms that govern lymphocyte differentiation.

MATERIAL AND METHODS

Animal and reagents:

Adult (8–12 months) male and female BALB/c mice were purchased from the Jackson Laboratory (Bar Harber, ME). Peanut agglutinin (PNA), from *Arachis hypogea*, either conjugated to fluorescein isothiocyanate (FITC–PNA) or coupled to agarose gel for affinity chromatography were obtained from Sigma Chemical Company (St. Louis, MO, USA). Endoglycosidase F from *Flavobacterium meningosepticum* (600 U/mg) and endoglycosidase H from *Streptomyces lividans* (25 U/mg) were purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN). Neuraminidase type V from *Clostridium perfringens* (1 U/mg) and all mono- and disaccharides were obtained from Sigma Chemical Company. All gel electrophoresis reagents and standards were from Bio-Rad (Richmond, CA).

Preparation of lymphocyte suspensions:

Thymuses, spleens and lymph nodes were excised from sacrificed mice and teased gently in 200 mM phosphate buffered saline (PBS), pH 7.2. Tissue debris were allowed to settle and cells in suspension collected and washed thrice in PBS followed by centrifugation at 300 g for 5 min at 4°C. For the isolation of lymphocytes from peripheral blood, aliquots of heparinized blood were centrifuged at 1800 rpm for 25 min on a gradient of Ficoll-hypaque, at the ratio of 1 : 3, and the lymphocyte-

rich layer at the interface aspirated and washed three times with PBS. Bone marrow lymphocytes were collected by cutting the heads and condyles off the femur bones and flushing them with PBS using a syringe with #40 gauge needle. The cells were washed once and lymphocytes fractionated on a gradient of Ficoll-hypaque. The final concentration of viable lymphocytes, obtained from the various organs, was adjusted by counting using trypan blue exclusion.

Direct fluorescent binding assays:
Viable lymphocytes of the different lymphoid organs (1×10^6 in 25 μl PBS, pH 7.2) were incubated separately with an equal volume of FITC-PNA (at 25 μg/ml) for 45 min at 4°C, then washed three times with PBS and examined using a Leitz Dialux microscope equipped with a ploemopak FITC-labeling blue excitation filter and lamp-housing (120 Z, 50 watt Hg) at a magnification of 640×. Assays were performed in triplicate and controlled by the substitution of FITC-PNA by FITC-conjugated bovine serum albumin (BSA), or by the inclusion of various mono- and disaccharides in the reaction mixture at various molar concentrations, as well as testing lymphocytes pre-treated with 0.02 U/100 μl of neuraminidase at 37°C for 1 h for desialylation. Percentage of positive cells was determined by counting an average of 300 lymphocytes and the reaction quantified according to the formula:

$$\text{PNA}^+ \text{ cells } \% = 100 \times \frac{\% \text{ of } + \text{ ve lymphocytes with FITC} - \text{PNA} - \% \text{ of } + \text{ ve lymphocytes with FTIC} - \text{BSA}}{100 - \% \text{ of } + \text{ ve lymphocytes with FITC} - \text{BSA}}$$

Paraffin-embedded sections of the thymus and the spleen were either stained with hematoxylin/eosin or dewaxed, rehydrated and stained with FITC-PNA (at 25 μg/ml) for 1 h at 37°C in a humidified chamber. Sections were washed in PBC, pH 7.2 and examined by light or fluorescent microscopy, and photomicrographs taken with a Wild photoautomat MPS 55 exposure meter and Kodak Tri-X-Pan films.

Pna-agarose affinity chromatography:
Lymphocyte cell-membranes were solubilized and extracted in 10 mM Tris/HCl, pH 8.0 containing 0.02% $NaNO_3$, 2 mM phenylmethylsulphonyl fluoride and 2% deoxycholate as previously described (Mansour *et al.* 1995b), and the protein content determined by the method of Lowry *et al.* (1951). Extracts of thymocytes and splenocytes were individually applied to a 3 ml column of PNA-agarose pre-equilibrated and washed with 20 mM Tris/HCl, pH 8.0 containing 0.02% $NaNO_3$ and 0.2% deoxycholate. Bound materials were, in both cases, eluted with the same buffer containing 200 mM D-galactose and the protein continuously monitored by measuring A_{280} nm The eluted fractions were freed of galactose by dialysis against water and freeze-dried prior to further analysis. To compare PNA-binding activities at different stages of solubilization and purification, a unit of activity was defined as the amount of extract or post-column fraction needed to give 50% inhibition of the binding of FITC-PNA to murine thymocytes. Thus, 1 ml of extract of which 25 μl

diluted 100-fold gave 50% inhibition of the binding, would contain 4000 units of activity.

Glycosidase treatments and polyacrylamide gel electrophoresis:
Freeze-dried fractions of post-PNA column eluates were reconstituted in either 50 μl of 100 mM phosphate, pH 6.1 containing 50 mM EDTA, 1% NP-40 and 100 mU of endo-F or 50 μl of 50 mM sodium citrate/phosphate, pH 5.5 containing 1% NP-40 and 10 mU of endo-H and incubated for 18 h at 37°C. Other fractions were reconstituted in 100 μl of 50 mM NaOH containing 1 M $NaBH_4$ and incubated for 24 h at 45°C. All treatments were terminated by freeze-drying followed by reconstitution in sample buffer and analysis by two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (2-D SDS-PAGE) as described by O'Farrell (1975). Samples were isoelectric focused for 18 h (8000 Vh) resolved in the second dimension with 12.5% slab SDS-PAGE (Laemmli 1970), stained with silver staining as described by Wray *et al.* (1981), and the gels photographed wet using Kodak Tri-X-Pan films.

Amino acid and carbohydrate composition analysis:
Reduced and alkylated fractions of thymic and splenic PNA-binding glycoproteins were independently hydrolyzed in 6 M HCl at 110°C for 24 and 72 h. Hydrolyzed samples were dried under vacuum and amino acid analysis performed in an automatic Jeol JLX-6AH amino acid analyzer equipped with an integrator. Half cystine values were obtained from the analysis of cystic acid residues resulting from performic acid oxidation. Neutral sugars, amino sugars and sialic acid were determined after methanolysis and trimethylsilylation in methanol/1.5 M HCl at 85°C for 18 h, on a Varion 3700 gas chromatograph using mannitol as an internal reference.

RESULTS

Binding of pna to murine lymphocytes:
The expression of a PNA receptor on murine lymphocytes was initially investigated by studying the reactivity of FITC-PNA towards lymphocyte suspensions, prepared from the different lymphoid organs in direct fluorescence assays. Regardless of the dilution of PNA utilized, the binding activity was always higher towards thymocytes than to lymphocytes of the other lymphoid organs. At a concentration of 25 μg/ml, 84% of thymocytes, 31% of peripheral blood lymphocytes, 20% of bone marrow lymphocytes, 11% of splenocytes and 8% of lymph node lymphocytes were directly stained with FITC-PNA (Table 1). Increasing the concentration of FITC-PNA did not alter the size of the stained population in any of the lymphoid organs, indicating that at the given concentration PNA receptor sites are fully saturated. In histological sections of the thymus and the spleen (Fig. 1), an intense fluorescent labeling was selectively observed on most of thymocytes of the cortical region, that typically comprise 80–90% of the total number of murine thymocytes, and thus confirm the data obtained from the FITC-PNA staining of thymocytes in suspension. In con-

Table 1. Distribution of PNA^+ lymphocytes in murine lymphoid organs.

	PNA^+ Cells (%)							
	Untreated*				Treated			
Cells	1	2	3	M ± SD	1	2	3	M ± SD
Thymocytes	85	82	86	84.3 ± 2.1	92	90	90	90.7 ± 1.2
Peripheral blood lymphocytes	36	25	32	31.0 ± 5.6	63	63	61	62.3 ± 1.2
Bone marrow lymphocytes	20	19	20	19·7 ± 0.6	35	37	31	34.5 ± 3.1
Splenocytes	15	6	13	11.3 ± 4.7	48	52	44	48.0 ± 4.0
Lymph node lymphocytes	16	3	6	8.3 ± 6.8	46	46	50	47.3 ± 2.3

* Fluorescence staining was conducted on cells that were either untreated or treated with neuraminidase as indicated in the Materials and Methods.

trast, no detectable surface fluorescent labeling was observed among medullary thymocytes (Fig. 1C). In the spleen, positive labeling with FITC-PNA was only evident in the white pulp, which is comprised of lymphoid aggregates concentrically arranged around central arterioles, with no evidence of positive labeling observed in the red pulp (Fig. 1D).

Alterations in the size of the PNA^+ lymphocytes populations were observed following treatment of lymphocytes with neuraminidase, which cleaves glycoprotein-associated terminal and sub-terminal sialic acid residues. These alterations were

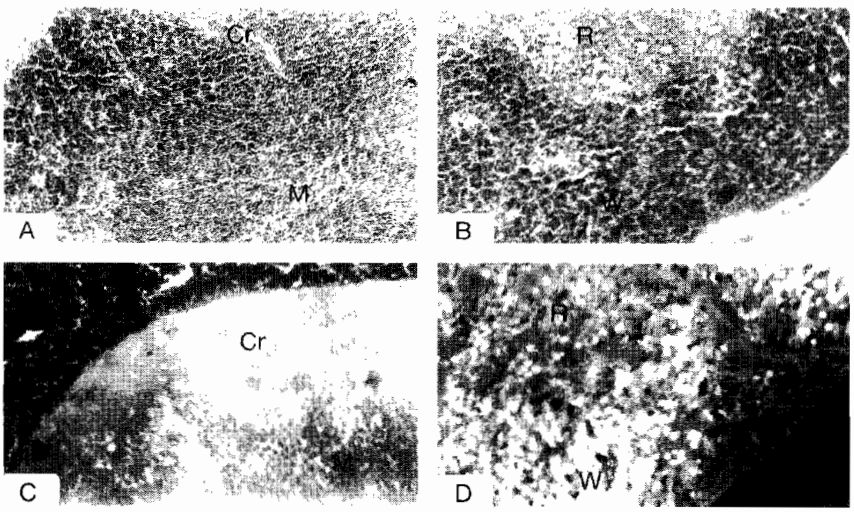

Fig. 1. Direct fluorescence localization of PNA^+ lymphocytes in the murine thymus and spleen. Sections of the thymus (A and C, X250) and spleen (B and D, X400) were either stained with hematoxylin/eosin (A and B, respectively) or dewaxed and stained with 25 μg/ml of FITC-PNA for 1 h at 37°C (C and D, respectively). Fluorescent labeling was selectively associated with cortical (Cr) and not medullary (M) lymphocytes in the thymus, and with the white pulp (W) and not the red pulp (R) in the spleen. No labeling was observed with FITC-BSA or with FITC-PNA in the presence of 50 mM galactose.

more pronounced among neuraminidase-treated lymph node lymphocytes and splenocytes, where more than a 4-fold increase in the PNA^+ population in both organs was observed (Table 1). On the other hand, only a 2-fold increase in PNA^+ lymphocytes was evident among peripheral blood and bone marrow lymphocytes, whereas the effect of the same treatment on thymocytes was marginal. In fluorescence assays conducted in the presence of different mono- or disaccharides, only glycans which contain galactose at their non-reducing terminal end were inhibitory to PNA-binding (Table 2). These sugars, as well as methylated and free galactose, were inhibitory at a relatively low molar concentration for PNA-binding to lymphocytes obtained from all lymphoid organs, indicating the specificity of PNA to terminal galactose residues expressed by a putative receptor in the thymus as well as periphral lymphoid organs.

Purification and characterization of murine thymic and splenic pna-binding glycoproteins:

PNA-binding glycoproteins associated with murine thymocytes and splenocytes were solubilized and purified by a procedure adapted from the method of Mansour & Cooper (1984) for purifying cell-surface differentiation markers in vertebrates. Thus, the murine PNA receptor was preliminarily extracted from crude membrane preparations, of both thymocytes and splenocytes, followed by solubilization in deoxycholate. The activity was monitored in both extracts by inhibiting the specific binding of FITC-PNA to glutaraldehyde-fixed thymocytes and a unit of activity defined as the amount of sub-cellular fraction needed to give 50% inhibition of the binding. Thus, a 1 ml of the extract, of which 25 μl diluted 100-fold, gave 50% inhibition of the binding would contain 4000 units of activity. As indicated in Table 3, extraction with deoxycholate was effective in releasing more than 75% of the activity in intact thymocytes and splenocytes at 2.4-fold and 2.25-fold purification, respectively.

Table 2. Inhibition of PNA binding by mono- and disaccharides.

Inhibitors	Inhibitor concentration (mM)*	Specificity factor
Galβ 1,3 GalNAc	3.3	1.0
Galβ 1,3 Ara	4.3	1.3
Galβ 1,4 Glc	4.8	1.4
Galβ 1,4 Man	5.1	1.5
Methyl-β-galactoside	6.2	1.9
Gal	9.8	2.8
L-Arabinose	15.2	4.6
Galβ 1,6 Glc	18.2	5.5
Gal NAc	21.5	6.5
Galacturonic acid	48.3	14.6
L-Rhamnose	75.2	22.8
Glc	104.0	31.5

* Assayed by inhibiting 50% of the binding of FITC-PNA at 25 μg/ml to murine lymphocytes.

Table 3. Purification of murine thymic and splenic PNA-binding glycoproteins.

Fraction	Thymocytes				Splenocytes			
	Protein (mg)	Activity units/fraction ($\times 10^{-3}$)*	Relative specific activity**	Yield (%)	Protein (mg)	Activity units/fraction ($\times 10^{-3}$)	Relative specific activity	Yield (%)
Cells (1.3×10^{10} thymocytes) (2.2×10^{10} splenocytes)	1225.3	27.2	1.0	100.0	3655.0	24.9	1.0	100.0
Crude membrane	620.8	25.3	1.8	93.0	1756.0	21.9	1.8	87.95
Deoxycholate extract	422.2	22.9	2.4	84.19	1229.2	18.8	2.25	75.50
Post-PNA column	0.32	14.3	2013.1	52.57	0.17	11.6	10,016.1	46.60

* Units of activity defined as the amount of protein in intact lymphocytes and sub-cellular fractions needed to give 50% inhibition of the binding of FITC-PNA to glutaraldehyde-fixed murine thymocytes.

** Specific activity is the number of units of activity per mg protein and is related to intact cells.

Deoxycholate extracts from thymocytes and splenocytes were independently loaded onto a PNA-agarose affinity column under conditions in which saturation was not reached. After washing, bound materials were eluted in a 200 mM galactose-containing deoxycholate buffer, pH 8.0. Details on the yield of activities and protein contents are summarized in Table 3. Approximately 62% of the activity in the thymocyte deoxycholate extract was recovered upon elution with galactose, and with only 0.08% of the protein, giving an overall yield of 53% and 2013-fold purification compared to the starting intact thymocytes. Under similar conditions, about 61% of the activity was recovered with 0.014% of the protein in the splenic deoxycholate extract, giving a yield of 47% and 10,016-fold purification compared to intact splenocytes. Comparisons of the purification factors, calculated for the PNA receptor in thymocytes and splenocytes, approached the estimation by direct fluorescence, which indicated that with respect to the content of this receptor, splenocytes would express several-fold less the amount expressed by thymocytes.

To judge the purity as well as the molecular characteristics of the two PNA-binding glycoproteins, post-PNA column eluates from thymocytes and splenocytes were subjected to 2-D SDS-PAGE. Under both non-reducing and reducing conditions, the thymic PNA-bound fraction was focused free of contaminants into a 24 kDa glycoproteins having two isomorphs of pI 5.0 and 5.2 (Fig. 2A). The splenic counterpart was, on the other hand, constituted by two PNA-binding acidic glycoproteins of 30 kDa and 28 kDa, each being composed of three isomorphs (Fig. 2B). The estimated pI values of the constituent charge variants of each of the two glycoproteins were 5.3, 5.4 and 5.6.

Differential n-linked glycosylations of thymic and splenic pna-binding glycoproteins: The nature of the glycan side-chains expressing the PNA-reactive determinant, as well as the structural interrelationship of the thymic and splenic PNA-binding glycoproteins, was investigated by testing the susceptibility of post-PNA column fractions to endo-F, endo-H and alkaline borohydride treatments and analyses by 2-D SDS-PAGE. As shown in Fig. 2C and D, treatments with endo-F resolved the microheterogeneous patterns of the thymic and splenic PNA-binding glycoproteins into a single homogeneous spot with an obvious shift in both molecular weight and charge. In both tissues, the endo-F treated PNA receptor focused as a single 21 kDa component with a pI of 5.6. Patterns observed with endo-H or alkaline borohydride treatments were essentially similar to those observed with the untreated thymic and splenic receptors (Fig. 2A & B), with no alterations in either the molecular weight or charge.

Given the known specificity of endo-F in cleaving linkages in the core of N-linked glycans [both complex and high-mannose types (Elder & Alexander 1982; Mansour 1995; 1996; Mansour *et al.* 1995a)] and based on the resistance of both thymic and splenic receptors to endo-H [selectively cleaves high-mannose type glycans (Traentino & Maley 1974)], the shift of about 3 kDa towards a basic pI observed with endo-F was consistent with the removal of a single complex-type glycan unit in the thymic PNA receptor. The shift of about 9 and 7 kDa observed with the 30 kDa and 28 kDa components of the splenic glycoproteins was consistent with the

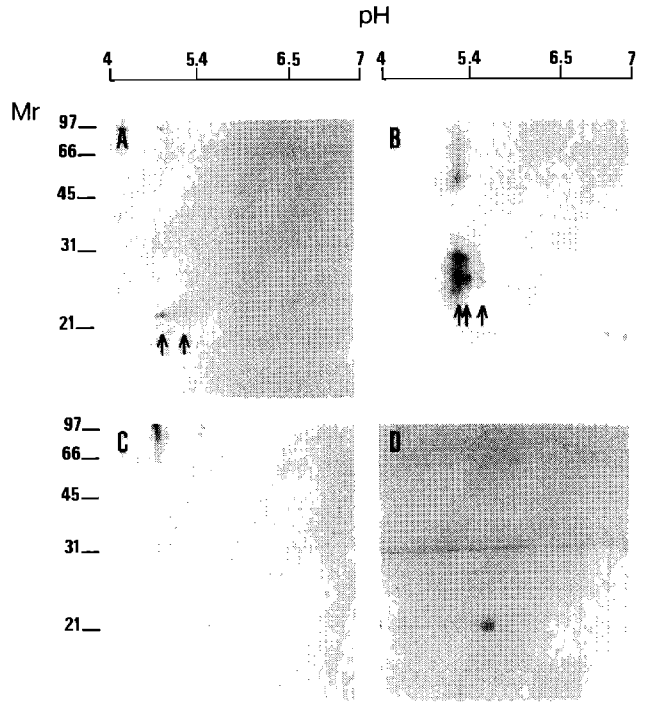

Fig. 2. Two-dimensional SDS-PAGE of glycosylated and deglycosylated PNA-binding glycoproteins in thymocytes and splenocytes. Purified PNA-binding glycoproteins in thymocytes and splenocytes were either untreated (A and B, respectively), or treated with endo-F (C and D, respectively) and analyzed by 2-D SDS-PAGE. Arrow-heads in (A) and (B) mark the constituent charge variants of the glycosylated PNA-binding glycoproteins in thymocytes and splenocytes, respectively. Also indicated are the positions of molecular weight (Mr $\times 10^{-3}$) standards.

removal of three and two complex glycan units, respectively. Inasmuch as both thymic and splenic receptors may lack O-linked glycans, as evidenced by their resistance to borohydride, a single polypeptide backbone of 21 kDa seems to be subjected to differential post-translational additions of one (thymic receptor) or two and/or three (splenic receptor) complex N-linked glycans of distinct structures, which account for the molecular weight and charge microheterogeneity of the glycosylated receptors in both lymphoid organs.

Amino acid and carbohydrate composition of thymic and splenic pna-binding glycoproteins:

Purified fractions (15 μg) of thymic and splenic PNA receptors were freed of deoxycholate by precipitation with 75% ethanol, dialyzed extensively against distilled water and analyzed for amino acid and carbohydrate compositions as described in the materials and methods section. Two analyses were performed on reduced and alkylated samples after 22 h and 72 h acid hydrolysis, respectively. Two other

analyses were performed after performic acid oxidation and 22 h acid hydrolysis. The compositions of both thymic and splenic receptors are given in Table 4, where for both molecules the serine and threonine values were extrapolated back to zero time of hydrolysis. For both purified receptors, the amino acid compositions were similar and the only statistically significant differences were those between the proline and glycine contents. The content of the other residues was, however, similar to the extent that any existing differences in the protein part of the two receptor molecules will only be clearly revealed by amino-acid-sequence analysis.

In contrast to the amino acid analysis, marked differences between the thymic and splenic PNA receptors were found in the carbohydrate composition. Table 4 shows the contents of neutral sugars, amino sugars and sialic acid, as determined by gas-liquid chromatography after methanolysis and tri-methylsialylation and expressed as residues per 100 amino acid residues for both receptor molecules. On a percentage basis, the thymic receptor revealed trace amounts of sialic acid, but more galactose residues, compared to those found in its splenic counterpart. The amounts of other amino and neutral sugars also differed by a factor of one or more between the thymic and splenic glycoproteins. Given that differences in composition are likely to reflect much larger differences in structure, the carbohydrate side-chains of thymocyte and splenocyte receptors may be completely unrelated, and thus confirm

Table 4. The amino acid and carbohydrate compositions* of thymic and splenic PNA-binding glycoproteins.

Amino acid/carbohydrate residues	Thymic	Splenic
Asx	11.0	10.8
Glx	8.7	9.1
His	3.9	4.2
Lys	7.3	7.0
Arg	7.0	7.2
Thr	9.9	8.1
Ser	7.0	7.2
Pro	2.9	5.3
Ala	2.8	2.6
Cys	3.0	3.0
Gly	6.4	5.2
Tyr	1.0	0.9
Val	8.2	7.7
Ile	3.7	3.9
Leu	11.1	11.4
Phe	5.1	5.4
Met	1.0	1.0
Fucose	0.8	1.6
Mannose	2.1	9.4
Galactose	3.1	1.9
Glucose	0.1	1.3
Glucosamine	2.1	3.5
Galactosamine	3.2	4.3
Sialic acid	0.4	6.2
Percentage by weight of carbohydrates	11.8	28.2

* Analysis were calculated as being equivalent to the mean number of each residue per 100 amino acid residues.

our data on the differential glycosylation patterns observed with the intact thymic and splenic PNA-binding glycoproteins (Fig. 2).

DISCUSSION

Carbohydrate moieties are involved in a number of developmental systems including early embryogenesis, where stage-specific oligosaccharide determinants are expressed (Cunningham 1986), the development of nervous tissue *via* the selective expression of cell adhesion molecules-associated glycans (Regan 1991), as well as abnormal neoplastic transformation as onco-fetal differentiation markers (Feizi 1985). Compelling evidence has suggested that the expression of developmentally-regulated glycan determinants may also have important implications in normal lymphocyte differentiation and development (Sharon 1983). In the present report, we expand on earlier experiments indicating the selectivity of PNA in probing glycoproteins associated with immature lymphocytes (Sharon 1983; Dall'Olio *et al.* 1991; Gillespie *et al.* 1993), and further demonstrate the expression of a novel low molecular weight receptor for this lectin, which exhibits distinctive structural differences in glycosylation patterns among murine thymocytes and peripheral lymphocytes in the spleen.

In accordance with previous observations (Reisner *et al.* 1976; London *et al.* 1981; Pulido & Sanchez–Madrid 1990), cortical immature lymphocytes, which represent the majority of thymocytes, were directly labeled with FITC-PNA, whereas more mature lymphocytes in the thymic medulla and in peripheral lymphoid organs were not. This labeling was selectively inhibited by the preferred disaccharide ligand of PNA, Galβ1,3 GalNAc (Sharon 1983), and to a lesser extent by other disaccharides with terminal galactose, and was expectedly enhanced by unmasking this ligand by desialylation. Under conditions employed for purification, multiple copies of the PNA ligand were expressed by distinct acidic charge variants of 24 kDa in the thymus and 30 kDa and 28 kDa glycoproteins in the spleen, which seemed to be distinct from PNA-binding glycoproteins previously identified with different strategies and included the 95 kDa component of rodent lymphocytes (Brown & Williams 1982), the CD 43 and CD 45 complexes of human thymocytes (DeMaio *et al.* 1986; Trowbridge & Thomas 1994), the 35 kDa molecule of lizard lymphocytes (Mansour *et al.* 1995a) as well as the spectrum of 40–160 kDa glycoproteins expressed by different malignant cell-lines (Flavell *et al.* 1989; Dall'Olio *et al.* 1991; Goulut-chassaing *et al.* 1992). Interestingly, comparisons based on 2-D gels of the glycosylated and deglycosylated forms of the thymic and splenic receptors, as well as their amino acid and carbohydrate compositions, were consistent with both receptors having structurally-similar 21 kDa polypeptide back-bones that differed substantially in their oligosaccharide side-chains. Both receptors were similar in lacking O-linked glycans and high mannose-type N-linked oligosaccharides, but were differentially glycosylated with one (thymic PNA receptor) or two and/or three (splenic PNA receptor) complex-type N-linked glycan units, which accounted for the heterodisperse patterns and the charge variability of the glycosylated forms of both receptors. Although the functional significance of the differential glycosylation of the thymic and splenic receptors is still to be resolved, the expression of the PNA disaccharide ligand on different complex-type glycans and the salient differences in

sialic acid contents imply a regulatory role for selective glycosyltransferases during lymphocyte maturation.

Various sialylated ligands, like members of the family of neuronal adhesion molecules (Regan 1991), are known to play important roles in intercellular signaling, adhesion and trafficing. On the other hand, addition of sialic acid can also inhibit cell-cell interactions by masking carbohydrate ligands and, thus, blocking receptor recognition (Schauer 1985). In this regard, it has been proposed that PNA receptors on cortical thymocytes are recognized by a galactose-specific lectin on thymic stromal cells, mediating the retention of immature thymocytes in the cortex (Sharon 1983). Indeed, a galactose-specific lectin has been identified in mouse thymus, which appears to be localized to thymic epithelium and which can agglutinate immature thymocytes but not mature thymocytes (Sharon 1983). Masking of lectin-binding sites by sialylation is postulated to inhibit interaction of thymocytes with cortical epithelium, allowing mature cells to migrate to the medulla.

Other changes in cell surface carbohydrates have been noted during various stages of T lymphocyte development. Changes in sialylation of N-linked oligosaccharides have been documented during T cell maturation (Lefrancois *et al.* 1985). In addition, mature T lymphocytes undergo characteristic alterations in the structure of cell surfaces O-linked glycans upon activation by interleukin-2 and anti-CD3 antibodies; these structural modifications are due to changes in the level of expression of two glycosyltransferase enzymes (Paulson *et al.* 1989; Gillespie *et al.* 1993). Along with these findings, observations reported in the present study may indicate that the regulation of glycosyltransferase expression adds another level of complexity to the mechanisms controlling the cell surface phenotype and may be an effective means of modulating cell-cell interactions during thymocyte maturation, selection, or migration.

REFERENCES

Brown, W.R.A. & Williams, A.F. 1982. Lympthocyte cell surface glycoproteins which bind to soybean and peanut lectins. Immunology **46:** 713–726.

Cook, R.G., Landolfi, N.F., Mehta, V., Leone, J. & Hoyland, D. 1987. Interleukin 2 mediates an alteration in the T200 antigen expression on activated B lymphocytes. Journal of Immunology **139:** 991–997.

Cunningham, B.A. 1986. Cell adhesion molecules: a new prospective on molecular embryology. Trends in Biochemical Sciences **11:** 423–426.

Dall'Olio, F., Malagolini, N., Distefano, G., Ciambella, M. & Serafini-Cessi, F. 1991. δ 2,6 sialylation of N-acetyllactosaminic sequences in human colorectal cancer cell lines. Relationship with nonadherent growth. International Journal of Cancer **47:** 291–297.

DeMaio, A., Lis, H., Gershoni, J.M. & Sharon, N. 1986. Identification of peanut agglutinin binding glycoproteins on immature thymocytes. Cellular Immunology **99:** 345–353.

Elder, J.H. & Alexander, S. 1982. Endo-β-N-acetylglucosaminidase F: Endoglycosidase from *Flavobacterium meningosepticum* that cleaves high mannose and complex glycoproteins. Proceedings of the National Academy of Science U.S.A. **79:** 4540–4549.

Feizi, T. 1985. Demonstration by monoclonal antibodies that carbohydrate structures of glycoproteins and glycolipids are onco-developmental antigens. Nature **314:** 53–57.

Flavell, D.J., Jones, D.B., Wright, D.H. 1989. Identification of peanut agglutinin binding glycoprotein restricted to Hodgkin's disease-derived cell line. Haematological Oncology **7:** 207–217.

Gillespie, W., Paulson, J.C., Klm, S., Pang, M. & Baum, L.G. 1993. Regulation of β 2,3-sialytransferase expression correlates with conversion of peanut agglutinin $(PNA)^+$ to $(PNA)^-$ phenotype in developing thymocytes. Journal of Biological Chemistry **268:** 3801–3804.

Goulut-Chassaing, C., Decastel, M., Tran, A.T., Tabarty, F. & Bourrillon, R. 1992. Identification of peanut agglutinin receptors related to the state of tumoral liver cell differentiation. Biochimie **74:** 101–108.

Laemmli, U. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685.

Lefrancois, L., Puddington, L., Machamer, C.E. & Beva, M.U. 1985. Acquisition of cytotoxic T lymphocyte-specific carbohydrate differentiatioll antigens. Journal of Experimental Medicine **162:** 1275–1293.

London, J., Thuillier, L., Garreau, F. & Petit, M. 1981. Study of rat lymphocytes by use of peanut agglutinin. Thymus **3:** 277–287.

Lowry, O.N., Rosebrough, N.J., Farr, A.L. & Randall, R.J. 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry **193:** 265–275.

Mansour, M.H. 1995. Evidence for a family of schistosome glycan-binding lectins in *Biomphalaria alexandrina*. Developmental and Comparative Immunology **19:** 365–376.

Mansour, M.H. 1996. Purification and characterization of SM37: A fucosyllactose determinant-bearing glycoprotein probed by a *Biomphalaria alexandria* lectin on adult male schistosomes. The Journal of Parasitology **82:** 586–593.

Mansour, M.H. & Cooper, E.L. 1984. Purification and characterization of *Rana pipions* brain Thy-1 glycoprotein. Journal of Immunology **132:** 2515–2523.

Mansour, M.H., Negm. H.I., Saad, A.H. & Taalab, N.I. 1995a. Characterization of *Biomphalaria alexandrina*-derived lectins recognizing a fucosyllactose-related determinant on schistosomes. Molecular and Biochemical Parasitology **69:** 173–184.

Mansour, M.H., Negm, H.I., Saad, A.H., Zahran, A.Y. & Badir, N. 1995b. Identification of peanut agglutinin-binding glycoproteins on lizard lymphocytes. Zoological Science **12:** 79–85.

Miyauchi, T., Muramatsu, H., Ozawa, M., Mizuta, T., Suzuki, T. & Muramatsu, T. 1982. Receptors for peanut agglutinin isolated from cell-lines of human Burkitt-lymphoma, lung squamous cell carcinoma and gastric signet ring cell-carcinoma. Gann **73:** 581–587.

O'Farrell, P.H. 1975. High resolution two-dimensional electrophoresis of proteins. Journal of Biological Chemistry **250:** 4007–4021.

Paulson, J.C., Weinstein, J. & Schachner, M. 1989. Tissue-specific expression of sialytransferases. Journal of Biological Chemistry **264:** 10931–10934.

Pulido, R. & Sanchez-Madrid, F. 1990. Glycosylation of CD45: carbohydrate composition and its role in acquisition of CD45RO and CD45RB T cell maturation-related antigen specificities during biosynthesis. European Journal of Immunology **20:** 2667–2671.

Regan, C.M. 1991. Regulation of neural cell adhesion molecule sialylation state. International Journal of Biochemistry **23:** 513–523.

Reisner, Y., Linker-Israeli, M. & Sharon, N. 1976. Separation of mouse thymocytes into two subpopulations by the use of peanut agglutinin. Cellular Immunology **25:** 129–134.

Schauer, R. 1985. Sialic acids and their role as biological masks. Trends in Biochemical Sciences **10:** 357–360.

Schulof, R.S., Naylor, P.H., Sztein, M.B. & Goldstein, A.L. 1987. Thymic physiology and biochemistry. Advances in Clinical Chemistry **26:** 203–292.

Sharon, N. 1983. Lectin receptors as lymphocyte surface markers. Advances in Immunology **34:** 213–298.

Tarentino, A.L. & Maley, F. 1974. Purification and properties of an endo-N-acetylglucosaminidase from *Streptomyces griseus*. Journal of Biological Chemistry **249:** 811–817.

Trowbridge, I.S. & Thomas, M.L. 1994. CD45: an emerging role as a protein tyrosine phosphatase required for lymphocyte activation and development. Annual Reviews in Immunology **12:** 85–115.

Wray, W., Boulikas, T., Wray, V.P. & Hancock, R. 1981. Silver staining of proteins in polyacrylamide gels. Analytical Biochemistry **118:** 197–203.

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التمييز التركيبي لمستقبل لأجلوتينين الفول السوداني ذو وزن جزيئي صغير في خلايا الفأر الليمفاويـــة

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خلاصة

تم في هذه الدراسة تحديد الصفات التركيبية وتنقية مستقبل جديد ذو وزن جزيئي صغير لأجلوتنين الفول السوداني وذلك من الخلايا الليمفاوية في الغدة الثيموسية والطحال في الفئران. ولقد تبين عن طريق صبغ الفلورسنس ارتباط هذا المستقبل بأسطح معظم الخلايا القشرية في الغدة الثيموسية وبعض من خلايا اللب الأبيض للطحال وكذلك الأعضاء الليمفاوية الطرفية الأخرى، كما تم تثبيط صبغ الفلورسنس بواسطة المركب الكربوهيدراتي Galb1, 3 GalNAc مما يؤكد خصوصية التفاعل تجاه مستقبل أجلوتنين الفول السوداني. ولقد تم تنقية هذا المستقبل من خلايا الغدة الثيموسية والطحال عن طريق الفصل الكروماتوجرافي بعامود أجاروز حامل لأجلوتنين الفول السوداني، وتحديد الصفات التركيبية بالمعالجة بالأنزيمات المختصة بالكربوهيدرات وكذلك البوهيدريد ثم التحليل بواسطة الفصل الكهربي ذو البعدين. وتشير النتائج الى وجود تركيب بروتيني موحد ذو وزن جزيئي 21 كيلو دالتون للمستقبل المنقى من كلا من الغدة الثيموسية والطحال، كما تشير الى تباين عدد وتركيب الوحدات الكربوهيدراتية ذات النوع المركب والمضافة للمستقبل في كلا من العضوين الليمفاوين. ولقد أكد تحليل الأحماض الأمينية والكربوهيدرات المكونة للمستقبل في كلا من الغدة الثيموسية والطحال هذا التباين في تركيب الكربوهيدرات وخاصة في محتوى حمض السياليك. ويشير البحث الى علاقة التباين التركيبي لهذا المستقبل في كلا من خلايا الغدة الثيموسية والخلايا الليمفاوية الطرفية بميكانيكية تطور وتميز الخلايا الليمفاوية.