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Identification of Peanut Agglutinin-Binding Glycoproteins on Lizard Lymphocytes

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ABSTRACT—The expression of PNA-binding glycoproteins on lizard lymphocytes was investigated by studying the reactivity of FITC-PNA towards lizard lymphocytes obtained from the different lymphoid organs. Direct immunofluorescence assays have demonstrated that the majority of lizard thymocytes (70%) and only a fraction of lymphocytes in the spleen, peripheral blood and bone marrow were PNA-positive. This positivity was selectively inhibited by galactose as well as lactose, indicating the specificity of binding. Putative PNA receptors were purified from lizard thymocytes and splenocytes by affinity chromatography on a PNA-Sepharose 4B column and resulted in fractions enriched 1,792-fold and 3,141-fold for the PNA-binding component expressed on lizard thymocytes and splenocytes, respectively. Analysis on reducing and non-reducing SDS-PAGE revealed that both thymic and splenic PNA-binding glycoproteins migrated as a single component of 35 KDa, with no evidence for the association into higher multimers in both tissues. Analyses for amino acid and carbohydrate compositions indicated that the thymic and splenic glycoproteins have similar amino acid composition and differed in the content of neutral and amino-sugars as well as sialic acid. The content of the latter residue was relatively higher in the splenic form of the receptor compared to its thymic counterpart, and was inversely correlated with the content of galactosyl residues in both forms of the receptor. The functional significance of PNA-binding glycoproteins during vertebrate evolution is discussed.

INTRODUCTION

Peanut agglutinin (PNA) binds to the disaccharide galactose β1,3 N-acetylgalactosamine (Gal β1,3 GalNAc), commonly found in O-glycosidically-linked glycan side-chains of glycoproteins [28]. Despite the wide distribution of this disaccharide, classic studies with PNA have demonstrated that the course of intra-thymic development is paralleled by alterations in the pattern of expression of thymocyte cell-surface carbohydrates. Thus PNA, which binds terminal galactose residues, stains immature, cortical cells brightly (PNA^+) but mature, medullary cells dimly (PNA^-) [24]. The apparent loss of PNA binding sites as thymocytes mature is beleived to be due to masking of terminal galactose residues with sialic acid [5, 24], which correlates with the increased level of sialyltransferase expression in medullary thymocytes relative to cortical thymocytes. Although the functional significance of this glycosylation change has not been elucidated, PNA-binding molecules have been implicated in the localization of normal as well as abnormal differentiation pathways of thymocytes as well as other cell types [25, 26, 28, 30].

On human thymocytes, PNA-binding molecules with variable degrees of sialylation have been identified as CD 45 and CD 43 [1, 5, 23]. On activated murine splenic B cells, PNA binds to T200, the murine homologue of CD 45 [3]. PNA-binding molecules on other blood cell types have been as yet not fully characterized, although PNA-binding glycoproteins have been detected on several malignant cell lines [6, 9, 26, 30] as well as the 160 KDa, molecule that has been demonstrated on Daudi cells [17]. Nonetheless, information regarding the possible occurrence of PNA-binding molecules in animal models other than mammals is very limited [29].

In the present study, we report for the first time on the purification and characterization of a 35 KDa glycoprotein that selectively bound to PNA and expressed on the cell-surface of lymphocytes of the lizard, *Chalcides ocellatus*. The significance of this finding resides in the implication of this glycoportein in the mechanisms that govern lymphocyte maturation and homing as an early event during the evolution of the vertebrate immune system.

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MATERIALS AND METHODS

Animals and reagents

Adult males and females of the lizard *Chalcides ocellatus* were collected from neighbouring gardens and fields in the vicinity of Cairo, Egypt. Lizards were maintained in wood and wire cages at a temperature range of 22–30°C. Water and live beetles were given *ad libitum*. A total of 600 lizards were used in the present study. Except where noted, all chemicals were reagent grade and purchased from Sigma Chemical Company (St. Louis, MO). CNBr-activated-Sepherose 4B, gel electrophoresis reagents and molecular weight standards were from Bio-Rad (Richmond, CA). Purified fractions of PNA and fluorescein isothiocyanate (FITC)-conjugated PNA were purchased from Sigma Chemical Company.

Preparation of lymphocyte suspensions

Thymuses and spleens were excised and gently teased apart in petri dishes in phosphate buffered saline (PBS, pH 7.2) using sterile disposable needles. Clumps were decanted and the cells washed thrice with PBS by centrifugation at 1,800 rpm for 5 min at 4°C. For the isolation of lymphocytes from peripheral blood (PBL), aliquots of heparinized blood were centrifuged at 1,800 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 (BML) were collected by cutting the heads and condyles of 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.

Fluorescent binding assays

The reactivity of FITC-conjugated PNA towards lizard lymphocytes was affected by direct immunofluorescence assays as described by Mansour *et al.* [15]. Briefly, 1×10^6 of glutaraldehyde-fixed lizard lymphocytes were incubated at 4°C for 45 min with 25 μl of FITC-labelled PNA (used at 1 mg/ml) and then washed three times with PBS containing 0.1% of bovine serum albumin (BSA). Controls were included in each assay system and involved FITC-labelled PNA +50 mM of various monosaccharides. Lymphocytes were scored microscopically at a magnification of 400–640× by using a Leitz Dialux microscope equipped with Ploemopak FITC labelling blue excitation and lamp housing 102 Z with 50 watt Hg. Lymphocytes exhibiting membrane staining from one bright point on the cell surface to complete ring reactions were counted as positive, whereas non-stained cells were scored as negative. Diffuse intracellular fluorescence was taken to indicate cell death, and such cells were discarded while counting. Percentage of positive cells was determined by counting an average of 300 lymphocytes and the reaction quantitated by calculating fluorescence indices percentage (Fl%) according to the formula:

$$Fl\% = \frac{\%\text{ of }+\text{ve lymphocytes in test} - \%\text{ of }+\text{ve lymphocytes in control}}{100 - \%\text{ of }+\text{ve lymphocytes in control}} \times 100$$

Solubilization of cell-membranes in deoxycholate

Lymphocytes were solubilized and extracted in 10 mM Tris/HCl (pH 8.0) containing 0.02% NaN_3, 2 mM phenylmethylsulphonyl fluoride (PMSF) and 2% sodium deoxycholate by sonic disruption followed by stirring on ice for 2 hr and then freezing overnight at −20°C. On thawing, the mixture was centrifuged at 100,000 g for 1 hr and the supernatant collected to give the solubilized cell membrane fraction. Protein content of extracts was determined by the method of Lowey *et al.* [13] using BSA in the same buffer as a standard.

PNA-Sepharose 4B affinity chromatography

PNA was dialysed against 50 mM sodium borate buffer (pH 8.0) and coupled to CNBr -activated Sepharose 4B at the ratio of 2 mg/ml swollen beads, and almost all was covalently bound. Before use, the column (2 ml) was pre-eluted and washed with 20 mM Tris/HCl (pH 8.0) containing 0.02% NaN_3, 0.2% deoxycholate and 200 mM D-galactose, (elution buffer) followed by three cycles of the same buffer without galactose (washing buffer). Both thymic and splenic extracts were individually applied to the column at 5 ml/hr and washed with washing buffer until the A 280 nm reading was reduced to that of the buffer. Bound materials were in both cases, eluted with 200 mM D-galactose buffer (elution buffer) and the protein continuously monitored by measuring A 280 nm readings. 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 fractions needed to give 50% inhibition of the binding of FITC-PNA to lizard thymocytes. Thus, 1ml of extract of which 25 μl diluted 100-fold gave 50% inhibition of the binding, would contain 4,000 units of activity.

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed on slab gels using the discontinuous buffer system of Laemmli [10] under both reducing and non-reducing conditions. Gels were electrophoresed using a Pharmacia vertical slab gel apparatus (Pharmacia Fine Chemicals, Sweden) at 25 mA constant current until the tracking dye (bromophenol blue) was within 0.5 cm of the gel edge. Gels were stained with silver stain according to Wray *et al.*[31] and photographed wet using Kodak Tri-X-Pan films. Marker proteins were phosphorylase B (94 KDa), BSA (67 KDa), ovalbumin (43 KDa), carbonic anhydrase (30 KDa), soybean trypsin inhibitor (20 KDa) and α-lactalbumin (14 KDa).

Reduction and alkylation of post-PNA column fractions

Freeze-dried, thymic and splenic post-PNA column fractions were individually reconstituted in 7 M guanidinium chloride-0.5 M Tris/HCl (pH 8.0) to give a concentration of 50 μg/50 μl. Dithiothreitol was added at room temperature for 1 hr, followed by iodoacetic acid for 30 min at 4°C in the dark. The reaction mixtures were quenched with excess dithiothreitol and excess reagents removed by dialysis against 0.1 M NH_4HCO_3.

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 hr. 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. Serine and threonine values were extrapolated back to zero time of hydrolysis, whereas the values of valine, isoleucine and phenylalanine were taken as found in the 72 hr hydrolysates. Values for the other amino acid residues were averaged over the two hydrolysis times. Neutral sugars, amino sugars and sialic acid were determined after methanolysis and trimethylsilylation in methanol/1.5 M HCl at 85°C for 18 hr, on a Varion 3,700 gas chromatograph using mannitol as an internal reference.

RESULTS

Binding of PNA to lizard lymphocytes

The possible expression of PNA-binding glycoproteins on lizard lymphocytes was investigated by studying the reactivity of FITC-PNA towards lymphocytes obtained from the different lymphoid organs of adult lizards in direct immunofluorescence assays. Regardless of the dilution of

TABLE 1. Distribution of PNA$^+$ lymphocytes in the different lymphoid organs of adult *Ch. ocellatus*

Treatment	Labeled lymphocytes (%±SD)[a]			
	Thymocytes	Splenocytes	PBL	BML
FITC-PNA[b]	72±3.1	40±7.1	27±5.1	22±2.5
FITC-PNA+ 50 mM Galactose	4±1.5	2±2.0	1±0.5	3±0.7
FITC-PNA+ 50 mM-Lactose	3±0.5	2±1.7	1±0.3	1±1.2
FITC-PNA+ 50 mM-Glucose	74±3.5	42±6.5	26±4.3	20±3.1
FITC-PNA+ 50 mM Sucrose	72±4.1	41±5.5	27±5.5	20±3.2
FITC-PNA+ 50 mM Fructose	75±4.5	39±6.5	28±3.5	23±2.3
FITC-PNA+ 50 mM Mannose	73±4.0	40±7.4	27±3.6	22±4.1

[a] Mean value of three separate experiments.
[b] Utilized at a concentration of 1 mg/ml.

PNA utilized, the binding activity was always higher towards thymocytes than to lymphocytes of the other lymphoid organs. At a dilution of 1 mg/ml, 70% of thymocytes, 40% of splenocytes, 27% of PBL and 20% of BML 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 were fully saturated. In reaction mixtures conducted in the presence of mono- or di- saccharides, only galactose and lactose, which contains galactose at its non-reducing terminal end, were inhibitory for PNA binding. Both sugars were equally inhibitory at 50 mM for lymphocytes obtained from all lymphoid organs, indicating the specificity of PNA to galactose residues expressed by PNA-binding molecules in the thymus as well as the periphery.

Solubilization and purification of PNA-binding glycoproteins

PNA-binding glycoproteins associated with lizard thymocytes and splenocytes were solubilized and purified by a procedure adapted from the method of Mansour *et al.*[14, 16] and Negm *et al.* [21, 22] for purifying cell-surface differentiation markers in lower vertebrates and invertebrates. Thus, the lizard PNA-binding glycoproteins were 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 fractions needed to give 50% inhibition of the binding. Thus, 1 ml of extract of which 25 μl diluted 100 -fold gave 50% inhibition of the binding would contain 4,000 units of activity. As indicated in Table 2, extraction with deoxycholate was effective in releasing more than 75% of the activity in intact thymocytes and splenocytes at 2.4-fold and 2.8-fold purification, respectively.

Deoxycholate extracts from thymocytes and splenocytes

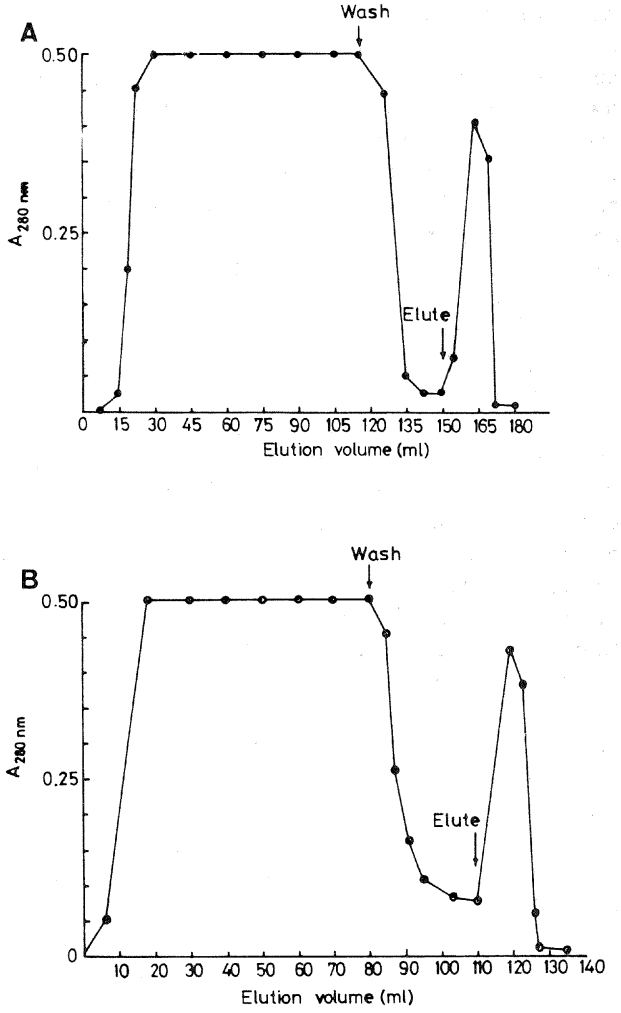

FIG. 1. Purification of the lizard PNA-binding glycoproteins by PNA affinity chromatography. Lizard thymic (115 ml, A) and splenic (80 ml, B) deoxycholate extracts were applied separately, to a PNA-Sepharose 4B affinity column, and bound materials eluted, in both cases, with 0.2 M glatactose in deoxycholate buffer. Fractions were collected and assayed for antigenic activities as indicated in the Materials and Methods. Protein content was continuously monitored at A 280 nm (●, ○).

TABLE 2. Purification of *Ch. ocellatus* thymic and splenic PNA-binding glycoproteins

Fraction	Lizard thymocytes				Lizard splenocytes			
	Protein (mg)	Activity Units/fraction ($\times 10^{-3}$)[a]	Relative specific activity[b]	Yield (%)	Protein (mg)	Activity Units/fraction ($\times 10^{-3}$)	Relative Specific activity	Yield (%)
Cells (1.3×10^{10} thymocytes) (2.2×10^{10} splenocytes)	1080.3	25.8	1.0	100.0	1955.0	24.9	1.0	100.0
Crude membrane	570.8	22.9	1.7	88.76	750.0	21.9	2.3	87.95
Deoxycholate extract	364.7	20.8	2.4	80.60	534.1	18.8	2.8	75.50
Post-PNA Column	0.32	13.7	1792.0	53.10	0.29	11.6	3141.0	46.60

[a] Unit of activity is defined as the amount of protein in intact lymphocytes and fractions needed to give 50% inhibition of the binding of FITC-PNA to glutaraldehyde-fixed lizard thymocytes.
[b] Specific activity is the number of units of activity per mg protein and is related to intact thymocytes.

were independently loaded onto a PNA-Sepharose 4B affinity column under conditions in which saturation was not reached and after washing, bound materials eluted with 0.2M galactose in 0.2% deoxycholate buffer as detailed in the materials and methods. The results of typical elution profiles for thymic and splenic extracts are depicted in Figure 1A and B, respectively, and details on the yield of activities and protein contents are summarized in Table 2. Approximately 66% of the activity in the thymocyte deoxycholate extract was recovered, upon elution with galactose, with only 0.09% of the protein giving an overall yield of 53% and 1,792-fold purification compared to the starting intact thymocytes. Under similar conditions, about 62% of the activity was recovered with 0.05% of the protein in the splenic deoxycholate extract giving a yield of 47% and 3,141-fold purification compared to intact splenocytes. A comparison of the purification factors, calculated for the PNA-binding glycoproteins in thymocytes and splenocytes approached the estimation by direct fluorescence, which indicated that with respect to the content of those glycoproteins, splenocytes expressed approximately twice less the amount expressed by thymocytes.

The purity of both thymic and splenic PNA-binding glycoproteins was analyzed and compared on SDS-PAGE under both non-reducing and reducing conditions (Fig. 2). Both purified fractions migrated as a single band under either reducing or non-reducing conditions, suggesting that, in both thymocytes and splenocytes, PNA-binding glycoproteins are expressed as a single polypeptide, with no evidence for a covalent or non-covalent association into higher multimers. It was noteworthy, however, that in contrast to the sharp band obtained with the thymic PNA-binding glycoprotein, the splenic counterpart appeared as a non-symetrical, diffuse doublet. This trailing phenomenon markes glycoproteins that express microheterogeneity in the carbohydrate side-chains and, accordingly, the splenic PNA-binding glycoprotein may be expected to express glycan variants, whereas its thymic counterpart would be more homogeneous with respect to these moieties. The estimated molecular weight for both the thymic and splenic PNA-binding glycoproteins was approximately 35,000.

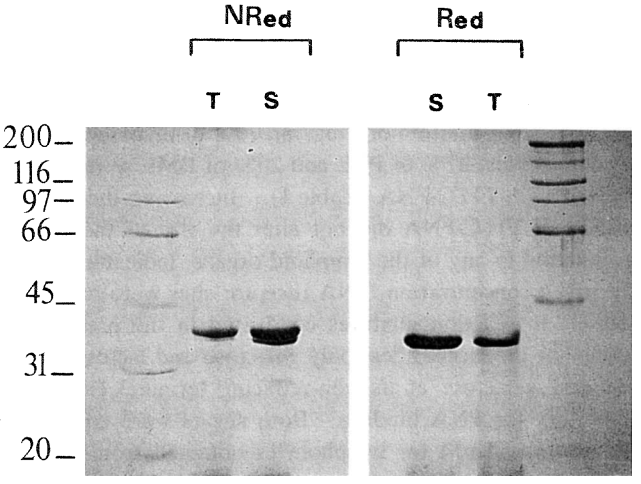

FIG. 2. SDS-PAGE of PNA-affinity purified non-reduced (NRed) and reduced (Red) thymic (T) and splenic (S) PNA-binding glycoproteins. The positions of molecular weight makers that were run simultaneously and silver-stained are indicated.

Amino acid and carbohydrate composition of thymic and splenic PNA-binding glycoproteins

Purified fractions (15 μg) of thymic and splenic PNA-binding glycoproteins were freed of deoxycholate 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 24 hr- and 72 hr-acid hydrolysis, respectively. Two other analyses were performed after performic acid oxidation and 24 hr-acid hydrolysis. Given in Table 3 are the compositions of both thymic and splenic PNA-binding glycoproteins, where for both molecules the serine and threonine values were extrapolated back to zero time of hydrolysis, and the valine, isoleucine and phenylalanine values were taken as found in the 72 hr- hydrolysate. For both purified glycoproteins, amino acid compositions were basically similar, except for

TABLE 3. The amino acid and carbohydrate composition* of thymic and splenic PNA-binding glycoproteins

amino acid/ carbohydrate residues	Thymic PNA-binding glycoprotein	Splenic PNA-binding glycoprotein
Asx	12.7	13.0
Glx	9.1	9.1
His	4.1	4.0
Lys	6.9	7.0
Arg	7.5	7.2
Thr	7.6	8.3
Ser	7.4	7.9
Pro	3.6	3.0
Ala	3.3	3.2
Cys	3.1	3.2
Gly	6.0	5.0
Tyr	2.0	2.0
Val	7.3	7.4
Ile	3.9	4.0
Leu	10.4	11.1
Phe	4.0	3.8
Met	1.1	0.8
Fucose	1.8	0.9
Mannose	11.9	9.4
Galactose	6.9	2.1
Glucose	0.6	1.1
Glucosamine	4.2	8.3
Galactosamine	4.6	3.1
Sialic acid	0.2	4.1
Percentage by weight of carbohydrates	29%	34%

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

differences observed in the threonine, leucine and glycine contents. The content of the other residues was however so similar that any differences, which may exist in the protein part of the two receptor molecules will only be clearly revealed by amino acid sequence analysis. Both molecules were notably more rich in hydrophilic residues than hydrophobic and non-polar residues and, in this respect, both molecules share a salient feature for most membrane glycoproteins.

In contrast to the amino acid analysis, marked differences between the PNA-binding glycoprotein of the thymus and spleen were found in the carbohydrate composition. Given in Table 3 are the contents of neutral sugars, amino sugars and sialic acid, as determined by gas chromatography after methanolysis and trimethylsilylation, and expressed as residues per 100 amino acid residues for both glycoproteins. On a percentage basis, the thymic PNA-binding glycoprotein revealed trace amounts of sialic acid compared to those found in its splenic counterpart. In contrast, galactose was present in small amounts in the splenic glycoprotein, but in larger amounts in the thymic molecule. The amounts of mannose, glucosamine and galactosamine also differed by a factor of one or more between thymic and splenic glycoproteins, while smaller differences on a percentage basis were found between fucose and glucose contents. Given that differences in composition are likely to reflect much larger differences in structure the carbohydrate side chains of thymocyte and splenocyte PNA-binding glycoproteins may be highly unrelated. However, from the summation of amino acid and carbohydrate residues, the percentage composition of carbohydrates was similar in both glycoproteins. Subsequently, given a molecular weight of 35,000 as determined by SDS-PAGE, then the size of the polypeptide portion would be about 24 KDa for the PNA-binding glycoproteins in both the thymus and the spleen.

DISCUSSION

Differentiation antigens of the lymphocyte cell surface are molecules which are expressed on lymphocytes at different maturational stages [2]. These antigens have been used to mark different types of lymphocytes and to determine relationships between different cells in ontogeny. At a more fundamental level, the antigens themselves may be involved in specific lymphocyte functions. In higher vertebrates, these determinants have been implicated in delineating T lymphocyte development as a consequence of both intrathymic and post-thymic events, in which hemopoietic progenitors are differentiated into precursors and effector cells [8, 27]. Although these features point to an evolutionary significance for the structural and functional aspects of these molecules in understanding the phylogeny of lymphocyte subpopulations, relatively limited information regarding the existense of lymphocyte differentiation antigens in lower vertebrates is available [4].

While the reptilian model has been unique for studying the immune potentialities as well as the migration and renewal cycles of lymphoid precursor cells and organs during the different seasons of the year [18], the characterization of reptilian lymphocyte differentiation antigens is still not fully explored [15, 19, 20]. The present study was thus conducted in a reptilian model to provide evidence for an early phylogenetic emergence of one of the classical cell-surface differentiation markers, namely the receptor for PNA. A homologue for PNA-binding glycoproteins was sought in the lymphoid tissues of the lizard *Chalcides ocellatus* by using serological and biochemical approaches. In direct immunofluorescence assays, the binding of a purified fraction of PNA towards lizard lymphocytes has demonstrated the existence of a distinct population, which readily expresses the receptor for the lectin. Variations in the size of the labeled population was, however, evident depending on the lymphoid tissue of origin. In consistence with observations among murine lymphocytes [24], the majority of lizard thymocytes (70%) and only a fraction of lymphocytes in the spleen, peripheral blood and bone marrow were readily labeled. Our observations in this regard confirm the dependencey of PNA-binding to lymphocytes on the species tested, since in

both rats and chickens, the majority of lymphocytes seemed to be PNA^+ in the thymus as well as the bone marrow and the periphery [1, 12, 29]. One important facet of our observation in ruling out non-specific labeling and in establishing the occurrence of PNA-binding glycoproteins in lizard lymphocytes, was the capacity of only galactose and galactose-containing sugars to inhibit the binding of PNA to thymic or peripheral lymphocytes. Thus, the selectivity of PNA in binding exposed galactose moieties, which is the typical mechanism that underlies the binding of the lectin to lymphocyte surfaces in higher vertebrates [28], seem to be similarly operative at this level of evolution.

A rapid two-step purification procedure, involving extraction and solubilization in non-ionic detergents followed by PNA affinity chromatography was adapted to isolate PNA-binding glycoproteins from lizard thymocytes and splenocytes. This procedure has been used successfully for purifying cell-surface determinants in phylogentically primitive species [14, 16, 21, 22], and in this study, has resulted in fractions enriched 1,792-fold and 3,141-fold for the PNA-binding components expressed on lizard thymocytes and splenocytes, respectively. The difference in the estimated degree of purification between thymocytes and splenocytes is consistent with the size-difference of the population positively stained with the fluoresceinated lectin in both tissues. On the other hand, the similarity in the yields may suggest that, on a per cell basis, equivalent amounts of the putative PNA-binding glycoproteins are expressed on thymocytes and splenocytes. Analysis by reducing and non-reducing SDS-PAGE revealed that both thymic and splenic post-PNA column fractions were constituted by a single 35 KDa component with no evidence for a covalent or non-covalent association into higher multimers in both tissues. In this respect, the reptilian PNA-binding glycoprotein seemed to be distinct from those identified in higher vertebrates, which included the 90 KDa component of rodent lymphocytes [1, 3], CD 43 and CD 45 complexes of human thymocytes [1, 5, 23] as well as the spectrum of PNA-binding glycoprotein range of 40–160 KDa expressed by various malignant cell lines [6, 9, 26, 30]. However based on the strict specificity of PNA to Gal β1,3 GalNAc, it seems plausible that similar PNA-binding sites would be carried by different polypeptide back-bones in different cell-types as well as in different animal models.

Comparisons based on amino acid and oligosaccharide compositions were consistant with the reptilian thymic and splenic PNA-binding glycoproteins having structurally-similar polypeptide back-bones that may differ substantially in their oligosaccharide side-chains. The content of sialic acid in particular was relatively higher in the splenic PNA-binding glycoprotein compared to the thymic counterpart, and was inversely correlated with the content of galactosyl residues in both molecules. This in turn suggested that the reptilian PNA-binding molecules may obey the general scheme that governs the modulation of the PNA phenotype depending on the maturational stage of lymphocytes. Among immature thymocytes in mammals, PNA binds with highest affinity to the disaccharide Gal β1,3 GalNAc, which is predominantly expressed on O-linked glycans [7, 12, 23]. This structure becomes masked by increased sialic acid additions to terminal galactose as well as subterminal GalNAc residues among mature lymphocytes in the thymic medulla and peripheral lymphoid organs [7]. In accordance with these observations, the differential sialylation of the reptilian PNA-binding glycoproteins may reflect the maturational status of developing thymocytes compared to effector lymphocytes in the spleen. It thus seems plausible that a continuous step-wise modulation of the thymic PNA-binding glycans, which may be controlled by the precise induction of different glycosyltransferases [7] could lead to the preferential exit of lymphocytes from the thymus and the eventual homing in the stroma of peripheral organs, thereby controlling the traffic of mature thymocytes and effector T lymphocytes in the periphery. These ideas should ultimately be tested in functional studies since they would establish the structural modulation of PNA-binding glycans as a common link of lymphocyte maturation at different levels of evolution.

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