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The glycoconjugate sugar residues of the sessile and motile cells in the thymus of normal and Cyclosporin-A-treated rats: lectin histochemistry

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Summary. It is well known that cell surface glycoconjugates play a determinant role in cellular recognition, cell-to-cell adhesion and serve as receptor molecules. T-lymphocytes are in strict contact with the thymic epithelial cells, which control their process of maturation and proliferation. On the other hand the normal maturation of the epithelial cells is believed to be induced by T-lymphocytes. For these reasons we have studied the glycoconjugates saccharidic moieties of the sessile and motile cells in the thymus of normal male albino Wistar rats and their changes following cyclosporin-A treatment, using a battery of seven HRP-lectins. Cytochemical controls were performed for specificity of lectin-sugar reaction. Some sections were pre-treated with neuraminidase prior to staining with HRP-lectins. Our results have demonstrated, in the control rats, a large amount and a variety of terminal and subterminal oligosaccharides within and/or on the epithelial thymic cells and in macrophages. After cyclosporin-A treatment, among the thymic epithelial cells, the subcapsular, paraseptal and perivascular cells showed the loss of some sugar residues, which characterized the same cells in the intact thymus. Some hypotheses are reported on the role played by the glycoconjugate sugar residues in control and cyclosporin-A treated rats.

Key words: Thymus, Sugar residues, CSA-treated rats

Introduction

T-lymphocytes and non-lymphoid cells constitute the microenviroment of the thymus. Non-lymphoid cells are classified into motile and sessile cells: the former are member of the mononuclear phagocytic system, the latter were represented by epithelial cells. All these cells play important roles in the thymocyte maturation (Janossy et al., 1983; Boyd et al., 1993) both through direct interaction among lymphocyte progenitors as well as by the production of lymphokines, growth factors and soluble hormones. It has also been shown that these cells are heterogeneous in morphology, functions, and immunophenotypic characteristics and that each subset occupies distinct locations within the thymus, thus defining a series of microenvironments related to the induction of different stages in T-lymphocyte development (for review see De Maagd et al., 1985; Milicévic and Milicévic, 1997).

Moreover, thymic non-lymphoid cells play a relevant role in the selection process by which only cells self unresponsive are allowed to survive, proliferate and leave the thymus as mature T-lymphocytes (Lo and Sprent 1986; Marrack et al., 1989). This selection process requires close and extended contacts among non-lymphoid cells and thymocytes, that are mediated by several adhesion molecules at both cell surfaces (Giunta et al., 1991).

Given that glycoconjugates play important roles in determining cell recognition, in maintaining cell adhesion as well as may serve as receptor molecules (Takata and Hirano, 1983; Fazel et al., 1989a,b; Gheri et al., 1993, 1994, 1997), we believed of some interest to study the oligosaccharidic component of the glycoconjugates of the thymic cells with particular regard to the motile and sessile non-lymphoid cells of the adult rat thymus and their changes after CSA administration.

Indeed ciclosporin-A (CSA) is a potent immunosuppressive drug widely used in clinics to prevent undesired cell-mediate immune responses such rejection of grafted organs and tissues, graft versus host and autoimmune disease (Weil, 1984; Feutren, 1993). CSA inhibits the activation of peripheral T-lymphocytes leading to immunosuppression (Hess and Colombani, 1987) and in the thymus causes an arrest in the thymocyte maturation, a marked atrophy of the thymic medulla, a loss of the thymic medullary epithelial cells, a depletion of interdigiting cells (IDC) with reduction of major histocompatibility complex (MHC) class II-

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positive cells (De Waal et al., 1996; Milicévic and Milicévic, 1997) therefore supporting a disturbance of clonal deletion during treatment.

Materials and methods

Twenty male albino Wistar rats, weighing 250-300 gr were used in this research. They were purchased from a commercial dealer and acclimatised for 5-7 days at 22-24 °C on a 12hr light/dark cycle before entering the experiment. A standard diet and water were available ad libitum.

Treatment

One group of ten rats was treated for 3 weeks (up to day 21) with a subcutaneous injection of 10 mg.Kg/ body weight/day of CSA (Sandoz Ltd, Basel, Switzerland) in cremophor as repository vehicle, and suspended in saline. Pharmacokinetic studies indicated that subcutaneous route allows steady and reproducible plasma levels of CSA to be obtained, with only slight variation, over the 24 h following administration (Wassef et al., 1985)

In the second group (controls) five rats were treated with saline alone and five with cremophor alone. At the end of the experiment (21 days) all the rats were killed by cervical dislocation between 10 and 11 AM and the thymus was removed for histological studies. Before sacrifice, blood samples were collected from the tail vein of treated rats in heparinized capillary tubes and immediately centrifuged to separate the plasma. CSA plasma levels were determined by radioimmunoassay using a commercial kit (Sandoz). At the end of experiments (three weeks) the rat weights were as follows: controls, 343±15 g; CSA treated, 273±26 g. The plasmatic concentration of CSA in rats treated with 10 mg. $Kg^{-1}.day^{-1}$ was 380±10 ng/ml.

The thymic tissue was fixed in buffered formaline, routinely processed for light microscopy, and 5µm-thick paraffin sections were cut. Some sections were stained with haematoxylin-eosin for general observations.

Lectin histochemistry

After hydration, the sections were treated with 0.3% hydrogen peroxide for 10 min to inhibit the endogenous peroxidase, rinsed in distilled water and washed with 1% bovine serum albumin (BSA) (Murata et al., 1983) in 0.1M phosphate-buffered saline (PBS) pH 7.2. The sections were then incubated for 30 min at room temperature in horseradish peroxidase-conjugated lectins (HRP-lectin conjugated) dissolved in phosphate-buffered saline (0.1M PBS pH 7.2, 0.1M NaCl, 0.1 mM $CaCl_2$, $MgCl_2$ and $MnCl_2$) and then rinsed three times in PBS. The optimal concentration for each lectin (Sigma Chemical Co., St. Louis, MO) which allowed maximum staining with minimum background was as following: DBA (*Dolichos biflorus*, binding specificity α-D-GalNAc) 25 µg/ml, SBA (*Glycine max*, binding specificity α/ß-D-GalNAc > D-Gal) 20 µg/ml, PNA (*Arachis hypogaea*, binding specificity D-Gal (ß1→3)-D-GalNAc) 25 µg/ml, ConA (*Canavalia ensiformis*, binding specificity α-D-Man > α-D-Glc) 50 µg/ml, WGA (*Triticum vulgare*, binding specificity $(\alpha\text{-D-GlcNAc})_n$ and sialic acid) 20 µg/ml, LTA (Lotus tetragonolobus, binding specificity α-L-Fuc) 25 µg/ml, and UEA I (*Ulex europaeus*, binding specificity α-L-Fuc) 25 µg/ml. Staining of the sites containing bound lectin-HRP was obtained by incubating the slides with PBS (pH 7.0), containing 3,3'-diaminobenzidine (DAB) (25 mg/100 ml) and 0.003% hydrogen peroxide, for 10 min at room temperature. Specimens were rinsed in distilled water, dehydrated using graded ethanol solutions, cleared in xylene and mounted in Permount.

Controls for lectin staining included: 1) substitution of unconjugated lectins for lectin-HRP conjugates; 2) exposure to HRP and substrate medium without lectin; 3) oxidation with 1% periodic acid for 10 min prior to lectin staining; and 4) exposure of sections to 10/12 µg/ml of each lectin-HRP conjugate containing 0.1M D-galactose, D-glucose, D-mannose, L-fucose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine and methyl-D-mannopyranoside.

Sialidase digestion

In some experiments sialic acid was removed by pretreating the sections for 18 hr at 37 °C in a solution of sodium acetate buffer 0.25M, pH 5.5, containing 0.1 unit/ml sialidase (neuraminidase Type X from *Clostridium perfrigens* (Sigma Chemical Co., St. Louis, MO)), 5.0 mM $CaCl_2$ and 154 mM NaCl, prior to staining with lectin-HRP conjugates. Controls containing the sialidase buffer without the enzyme were also prepared.

Statistical analysis

For estimation of macrophage number in normal and CSA-treated thymus, ten sections of ten different rats belonging to the two groups were evaluated using Image Pro Plus program (version 4.0-Media Cybernetics) for image analysis.

Statistical analysis was performed using the t test for paired samples.

Results

Morphological remarks

The thymus of control rats displayed normal histological features.

The most significant changes in the thymus of CSA-treated rats were represented by a marked atrophy of the thymic medulla, whereas the cortex appeared well preserved and characterized by numerous macrophages

containing pyknotic nuclear debris, which gave to this thymic region a "starry-sky" appearance, and by a cytoreticulum of epithelial cells denser than that of controls.

Interdigitating reticular cells (IRC) and medullary epithelial cells were strikingly reduced in number. Hassall's bodies within the residual of the thymic medulla were never detected.

Control rats (Tables 1 and 2)

PNA

PNA lectin reacted with the surface and with small cytoplasmic granules of the subcapsular cells (Fig. 1). The other cortical epithelial cells, star-shaped, showed reactivity at the cell surface and at granules within the

Table 1. Lectin reactivity in the thymus of control rats and in CSA-treated rats.

	PNA	SBA	DBA	ConA	LTA	UEAI	WGA
Subcapsular	**S Cgr>**	**S Cgr>**		**S Cgr***	**S C** S C	**S Cgr** S *	**S C<** S*
Paraseptal		**S Cgr>**		**S Cgr***	**SC** SC	**S Cgr***	**S Cgr<** S*
Perivascular		**S>** S					**S C<** S*
Other cortical epithelial cells	**S Cgr** S Cgr	**Cgr** Cgr		**Cgr** Cgr	**C***	**Cgr** Cgr	**S Cgr** S Cgr
Large cortico-medullary cells	**S Cgr** S Cgr	**Cgr**		**Cgr**		**S Cgr** S Cgr	**S Cgr** S Cgr
Spindle shaped medullary cells	**S Cgr**	**Cgr**		**Cgr**			**S Cgr**
T-lymphocytes	 +(a.n.)	 +(a.n.)		**+ (c&m)** +§			**+** +§
Macrophages	**+** +(a.n.)	**+** +	**+** +	**+** +			**+** +
Hassall's corpuscles	**+**	+	+		**+**		+
Mastocytes	 +(a.n.)	 +		**+** +	**+** +	 +	

Legend: in bold, control rats; S, surface of the cells; Cgr, cytoplasmic granules; C, diffuse cytoplasmic reactivity; *, few cells; >, the reaction increases after neuraminidase digestion; <, the reaction decreases after neuraminidase digestion; +, positive reaction; c&m, cortical and medullary T-lymphocytes; (a.n.), after neuraminidase ; §, weaker reactivity

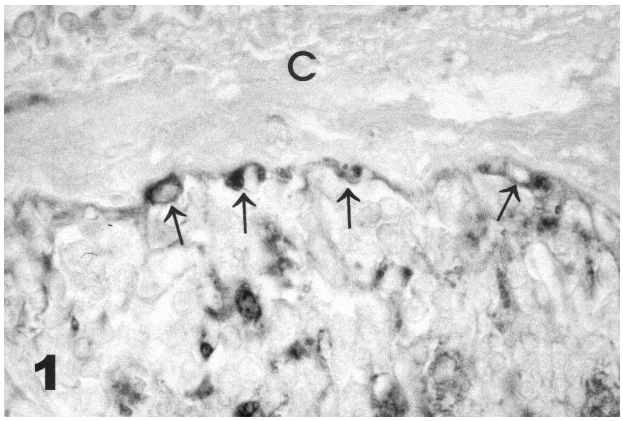

Fig. 1. HRP-PNA. Thymus of the control rats. Subcapsular epithelial cells (arrows) exhibit PNA reactivity. C: capsule. x 900

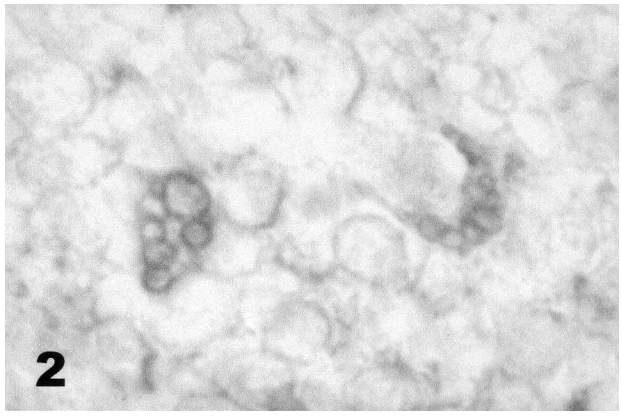

Fig. 2. HRP-PNA. Thymus of the control rats. Large reactive granules are observable within the cortical epithelial cells. x 1,100

cytoplasm of cellular body and of their cytoplasmic processes. Some epithelial cells, located in the middle part of the cortex showed large reactive granules frequently arranged like a “bunch of grapes” (Fig. 2). Conversely, the cytoplasmic reactive granules were small in the cells localised in the outer and deeper cortex. PNA reactivity was also detected in large epithelial cells located at the cortico-medullary boundary and within the medulla interspersed with smaller cells often arranged in groups. Macrophages and a few Hassall’s corpuscles were also reactive. An increase in reactivity after neuraminidase treatment was observed only in the subcapsular cells.

Table 2. Sugar residues identified in the sessile and motile thymic cells

	CONTROL RATS	CSA-TREATED RATS
Subcapsular cells	D-Gal (ß1→3)-D-GalNAc	-
	ß-D-GalNAc	-
	α-D-Man	-
	α-L-Fuc (LTA)	α-L-Fuc (UEAI)
	α-L-Fuc (UEAI)	α-L-Fuc (LTA)
	$(\alpha\text{-D-GlcNAc})_n$	(α-D-GlcNAc)n
	sialic acid	-
Paraseptal cells	ß-D-GalNAc	-
	α-D-Man	-
	α-L-Fuc (LTA)	α-L-Fuc (LTA)
	α-L-Fuc (UEAI)	-
	$(\alpha\text{-D-GlcNAc})_n$	$(\alpha\text{-D-GlcNAc})_n$
	sialic acid	
Perivascular cells	ß-D-GalNAc	ß-D-GalNAc
	$(\alpha\text{-D-GlcNAc})_n$	$(\alpha\text{-D-GlcNAc})_n$
	sialic acid	-
Other cortical epithelial cells	D-Gal (ß1→3)-D-GalNAc	D-Gal (ß1→3)-D-GalNAc
	ß-D-GalNAc	ß-D-GalNAc
	α-D-Man	α-D-Man
	α-L-Fuc (LTA)	-
	α-L-Fuc (UEAI)	α-L-Fuc (UEAI)
	$(\alpha\text{-D-GlcNAc})_n$	$(\alpha\text{-D-GlcNAc})_n$
Large corticomedullary cells	D-Gal (ß1→3)-D-GalNAc	D-Gal (ß1→3)-D-GalNAc
	ß-D-GalNAc	-
	α-D-Man	-
	α-L-Fuc (UEAI)	α-L-Fuc (UEAI)
	$(\alpha\text{-D-GlcNAc})_n$	$(\alpha\text{-D-GlcNAc})_n$
Spindle shaped cells	D-Gal (ß1→3)-D-GalNAc	-
	ß-D-GalNAc	-
	α-D-Man	-
	$(\alpha\text{-D-GlcNAc})_n$	-
T-lymphocytes	α-D-Man	D-Gal (ß1→3)-D-GalNAc
	$(\alpha\text{-D-GlcNAc})_n$	ß-D-GalNAc
	-	α-D-Man
	-	$(a\text{-D-GlcNAc})_n$
	-	sialic acid
Macrophages	D-Gal (ß1→3)-D-GalNAc	D-Gal (ß1→3)-D-GalNAc
	ß-D-GalNAc	ß-D-GalNAc
	α-D-GalNAc	α-D-GalNAc
	α-D-Man	-
	$(\alpha\text{-D-GlcNAc})_n$	$(\alpha\text{-D-GlcNAc})_n$
	-	sialic acid
Hassall’s corpuscles	D-Gal (ß1→3)-D-GalNAc	-
	ß-D-GalNAc	-
	α-D-GalNAc	-
	α-L-Fuc (UEAI)	-
	$(\alpha\text{-D-GlcNAc})_n$	-
Mastocytes	-	D-Gal (ß1→3)-D-GalNAc
	-	ß-D-GalNAc
	α-D-Man	α-D-Man
	α-L-Fuc (LTA)	α-L-Fuc (LTA)
	-	α-L-Fuc (UEAI)

SBA

The cell surface and the cytoplasmic granules of the subcapsular (Fig. 3) and paraseptal cells reacted with SBA, while only the cell surface of the perivascular cells (Fig. 4) was marked by this lectin. Following neuraminidase treatment, SBA reactivity was enhanced.

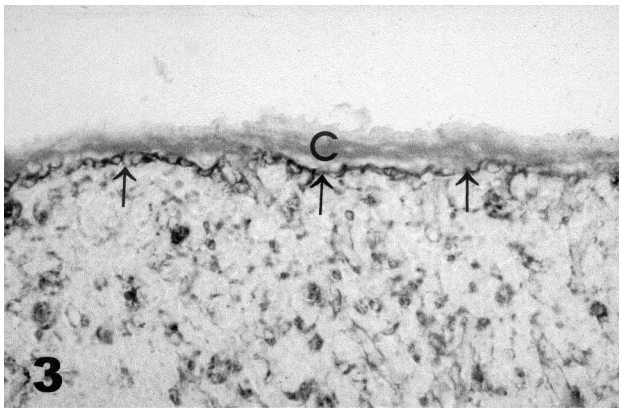

Fig. 3. HRP-SBA. Thymus of the control rats. SBA-reactive subcapsular (arrows) an cortical cells are seen. C: capsule. x 180

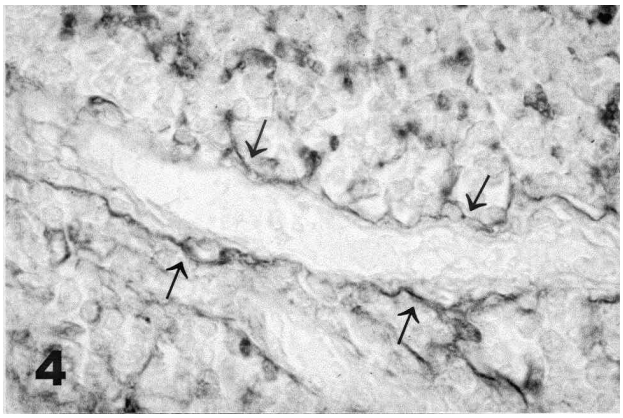

Fig. 4. HRP-SBA. Thymus of the control rats. At the cortico-medullary boundary perivascular cells (arrows) show reactivity with SBA lectin. x 900

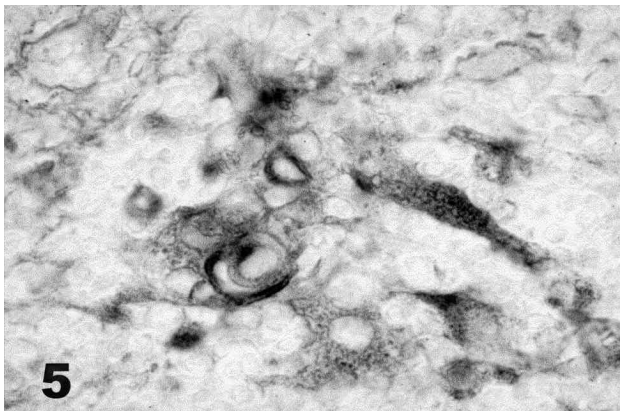

Fig. 5. HRP-SBA. Thymus of the control rats. Within the thymic medulla SBA-reactive large cells and a Hassal's corpuscle show lectin reactivity. x 900

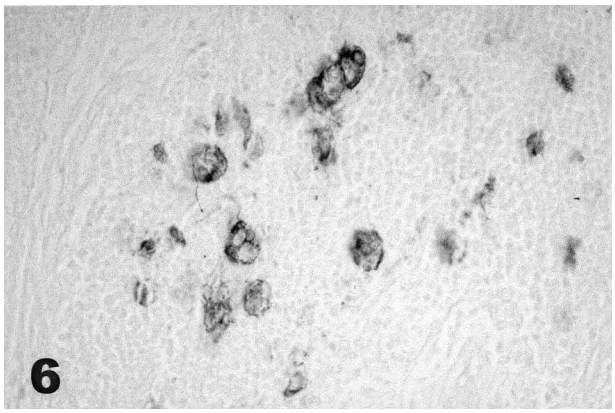

Fig. 6. HRP-DBA. Thymus of the control rats. Thymic cortex. Only macrophages show DBA reactivity. x 360

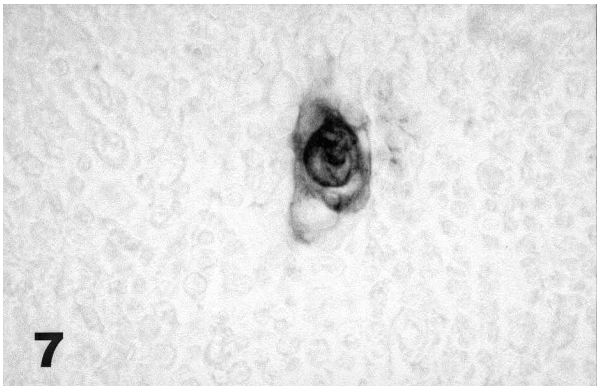

Fig. 7. HRP-DBA. Thymus of the control rats. Thymic medulla. A Hassal's corpuscle shows reactivity with DBA. x 900

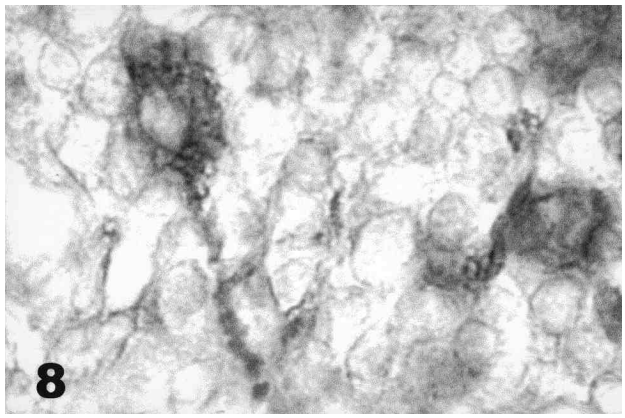

Fig. 8. HRP-ConA. Thymus of the control rats. The cortical epithelial cells and their cytoplasmic processes strongly react with ConA. T-lymphocytes react more weakly with this lectin. x 1,100

In the other cells of the thymic parenchyma (cortex, cortico-medullary boundary, medulla) the SBA reactivity was detected only at the cytoplasmatic granules (Fig. 5). The rare Hassall's corpuscles and cytoplasm of macrophages reacted with SBA.

DBA

This lectin only reacted with the cytoplasm of the macrophages and with the Hassall's corpuscles (Figs. 6, 7).

ConA

ConA reactivity was detected at the surface and the cytoplasm of a few scattered subcapsular and paraseptal cells. Within the cortex minute granules of the cellular body of the epithelial cells (Fig. 8) displayed reactivity for this lectin and only rarely granules located in the cellular prolongation were reactive. The large cells positioned in the cortico-medullary boundary and the spindle-shaped cells in the thymic medulla showed reactivity with this lectin throughout the cytoplasm. The cytoplasm of the macrophages, the surface of the cortical and medullary T-lymphocytes and the granules of the mastocytes reacted with ConA.

LTA

LTA reactivity was detected at the surface and in the cytoplasm of subcapsular and paraseptal epithelial cells (Fig. 9). Within the thymic cortex only very few cells showed weak cytoplasmic reactivity with LTA.

The Hassall's corpuscles and the capsular mastocytes reacted with this lectin.

UEAI

UEAI reacted at the cell surface and at cytoplasmic granules of the subcapsular and paraseptal cells. UEAI-reactive granules, found in the cytoplasm and in the

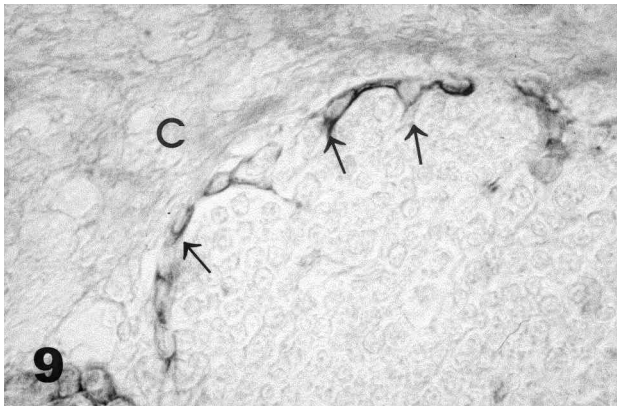

Fig. 9. HRP-LTA. Thymus of the control rats. Reactivity is observable at the subcapsular cells (arrows). C: capsule. x 900

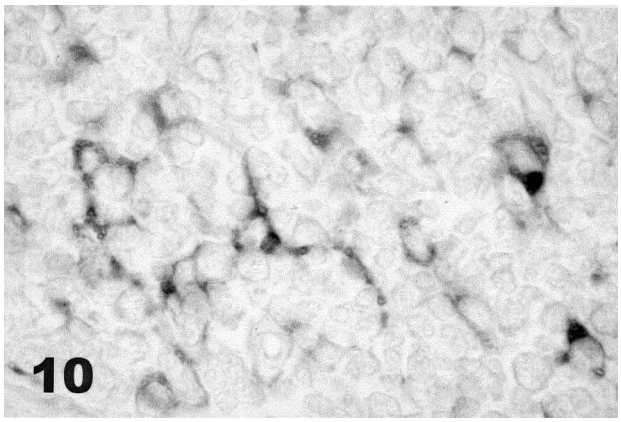

Fig. 10. HRP-UEAI. Thymus of the control rats. The cortical epithelial cells and their cytoplasmic processes are characterised by UEAI-reactive granules. x 900

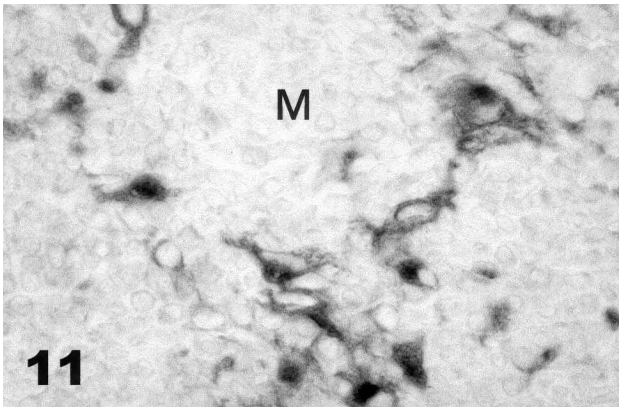

Fig. 11. HRP-UEAI. Thymus of the control rats. Large epithelial cells, positioned at the cortico-medullary boundary, show strong reactivity with UEAI. M: medulla. x 900

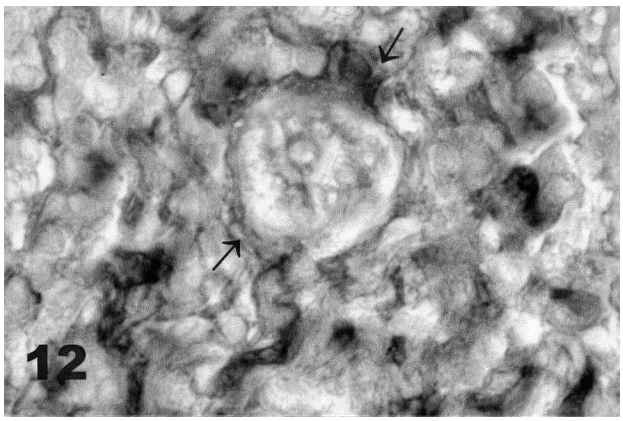

Fig. 12. HRP-WGA. Thymus of the control rats. Perivascular cells (arrows) show reactivity with WGA. x 900

cellular processes were detected at the cortical epithelial cells (Fig. 10). At the cortico-medullary boundary, groups of large epithelial cells showed UEAI reactivity at the cell surface and in cytoplasmic granules.
These cells, disposed to form an almost uninterrupted chain, seemed to separate the thymic cortex from the medulla (Fig. 11).

WGA

The cell surface and the cytoplasm of the subcapsular, paraseptal and perivascular epithelial cells reacted with WGA (Fig. 12). In these cells, a little decrease in WGA reactivity was observed after neuraminidase digestion.

The other cortical epithelial cells showed thin WGA-reactive cytoplasmic granules. The large epithelial cells located at the cortico-medullary boundary reacted intensely with WGA both at their cell surface and in the cytoplasmic granules. WGA reactivity was also seen in spindle-shaped cells within the thymic medulla.

Macrophages, T-lymphocytes and the Hassall's corpuscles reacted with WGA.

No difference in lectin reactivity was detected among the control rats and the cremophor-treated rats.

Cyclosporin-A-treated rats (Tables 1 and 2)

PNA

After the daily administration of 10mg/Kg of CSA no reactivity with PNA was detected in the subcapsular (Fig. 13), paraseptal or perivascular cells while the other cortical epithelial cells were reactive on the surface and characterized by thin positive granules both in the body and in the thinner and longer cytoplasmic processes (Fig. 14). The large epithelial cells of the cortico-medullary boundary, disposed to form a ring around the residual of the thymic medulla, reacted intensely with this lectin.

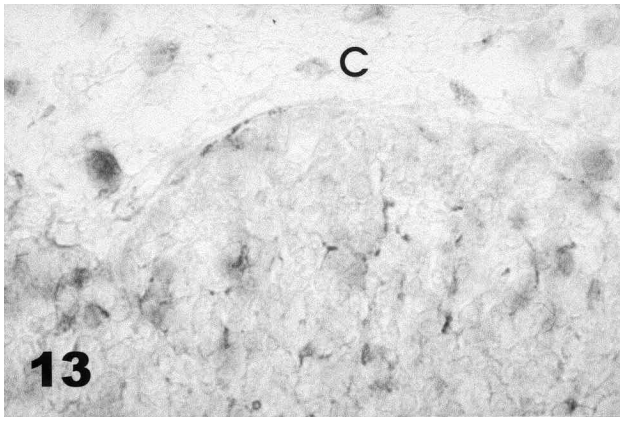

Fig. 13. HRP-PNA. Thymus of the CSA-treated rats. After CSA treatment, PNA reactivity is no more observable at the subcapsular cells. x 900

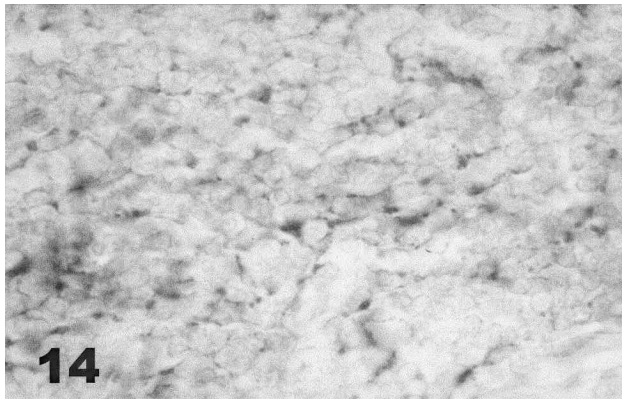

Fig. 14. HRP-PNA. Thymus of the CSA-treated rats. Thin, reactive granules are seen within the cortical epithelial cells and within their processes. x 900

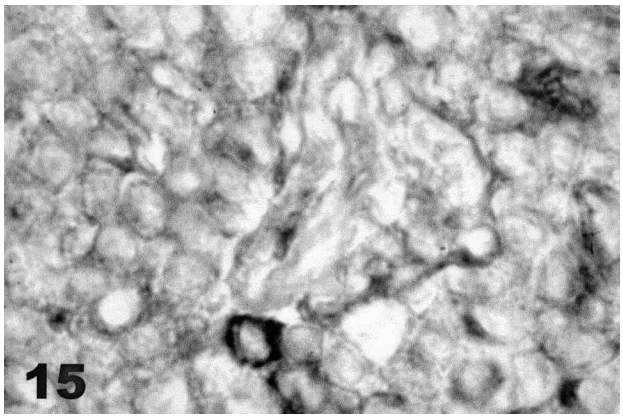

Fig. 15. Neuraminidase-HRP-PNA. Thymus of the CSA-treated rats. Following neuraminidase digestion PNA reactivity is also observable at the T-lymphocytes. x 900

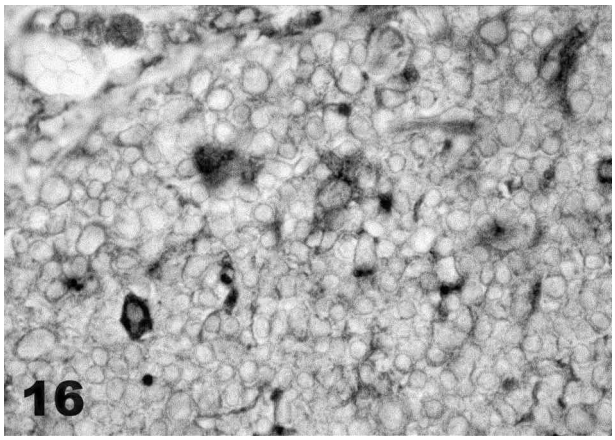

Fig. 16. HRP-ConA. Thymus of the CSA-treated rats. The epithelial cells and the T-lymphocytes show reactivity with ConA. x 900

After neuraminidase treatment no changes in reactivity was detected.

After neuraminidase treatment, PNA reactivity was also detected in the macrophages, T-lymphocytes (Fig. 15) and mastocytes.

SBA

Reactivity with this lectin was observed only at the surface of the perivascular epithelial cells and in the cytoplasmic granules of the other cortical epithelial cells which displayed long, thin cellular processes. After neuraminidase treatment no changes in reactivity were detected.

Macrophages maintained their SBA reactivity and the mastocytes appeared reactive with this lectin. T-lymphocytes appeared reactive with this lectin only after neuraminidase treatment.

DBA

DBA reactivity was only detected in the macrophages, which increased in number five times with respect to the controls (114±27 vs. 556±45; $p<10^{-5}$).

ConA

Cortical ConA-reactive epithelial cells, lesser in number than those observed in normal rats, were detected (Fig. 16).

Macrophages, mastocytes and T-lymphocytes reacted with this lectin.

LTA

Only few subcapsular and paraseptal (Fig. 17) cells reacted with this lectin; reactivity with LTA was also observed at the granules of the mastocytes.

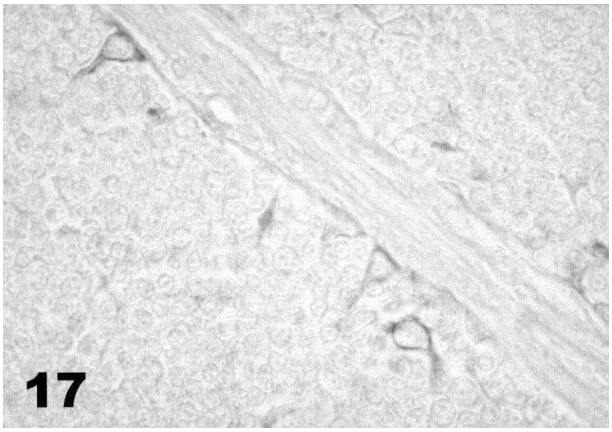

Fig. 17. HRP-LTA. Thymus of the CSA-treated rats. Reactivity with LTA is observable in scattered paraseptal cells. x 900

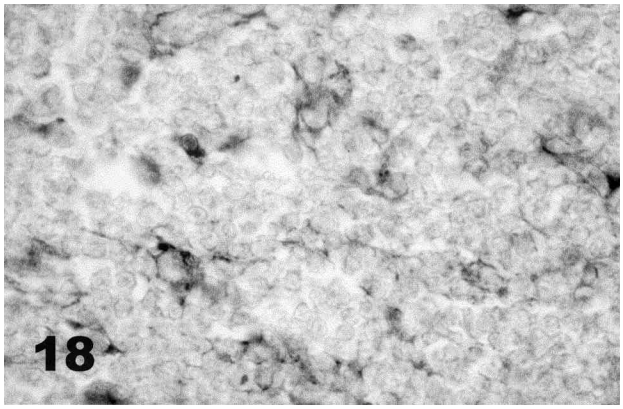

Fig. 18. HRP-UEAI. Thymus of the CSA-treated rats. The cortical epithelial cells and their long, thin cytoplasmic processes react with this lectin. x 900

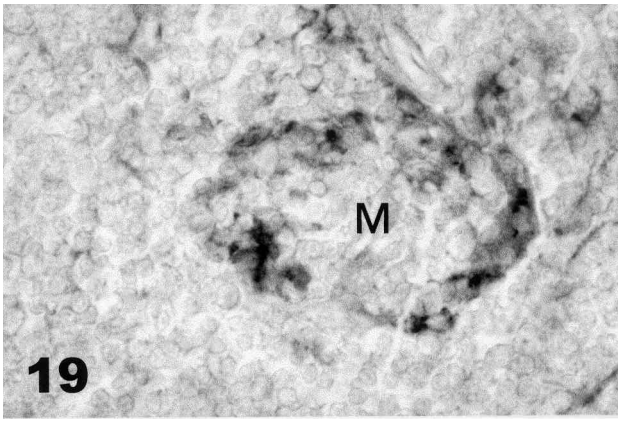

Fig. 19. HRP-UEAI. Thymus of the CSA-treated rats. Large UEAI-reactive epithelial cells are observable around the residual of the thymic medulla (M). x 900

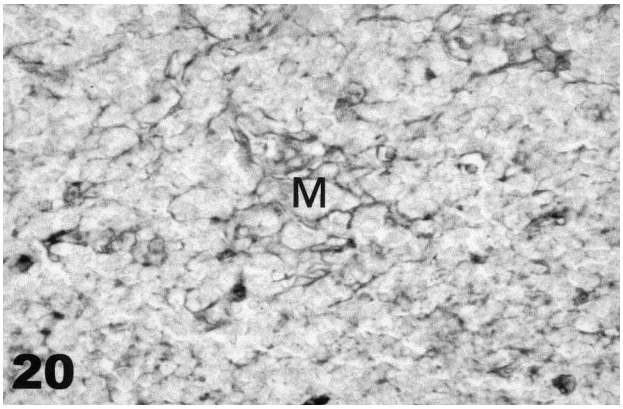

Fig. 20. HRP-WGA. Thymus of the CSA-treated rats. Within the residual of the thymic medulla (M) a network of the long, reactive cellular processes is observable. x 360

UEAI

UEAI reacted with the surface of few subcapsular epithelial cells. The other cortical epithelial cells showed reactivity in small granules within the cellular body and within the long cellular processes (Fig. 18). The large epithelial cells located between the cortex and the residual of the medulla were reactive with this lectin (Fig. 19). The granules of mastocytes reacted with UEAI.

WGA

The reactivity with this lectin was maintained at the surface of few subcapsular, paraseptal, perivascular cells, and at the surface, and cytoplasmic granules of the other cortical epithelial cells. The large epithelial cells located at the cortico-medullary boundary gave rise with their long cytoplasmic processes to a network within the residual medulla (Fig. 20). After neuraminidase treatment no changes in reactivity were detected.

WGA reactivity was maintained either at the macrophages or at the surface of the T-lymphocytes. In these latter WGA reactivity was weaker than that observed in control rats.

Discussion

To our knowledge, no studies have been carried out on the oligosaccharidic content of the glycoconjugates of the thymic non-lymphoid cells, with the only exception of a research which demonstrated how fucose-specific lectins (UEAI and TPA) bound medullary epithelial cells in murine thymuses (Farr and Anderson, 1985). On the other hand the important role played by glycoconjugates in cell to cell recognition and adhesion, now widely accepted, is particularly evident in the thymus, where the development of the medullary precursor into fully functionally competent T-lymphocytes occurs by direct contact with non-lymphoid cells of thymic parenchyma. It is also believed that the normal maturation of the non-lymphoid cells is in some way induced by their strict adhesion with the T-lymphocytes, which exert a feedback influence on non-lymphoid cells controlling their integrity (Surh et al., 1992; Van Ewijk et al., 1994) and organisation (Goverman et al., 1997).

Our data, obtained using a battery of seven different lectins, demonstrated in the thymic epithelial cells of the control rats, a large amount of glycoconjugates with a variety of terminal and subterminal sugar residues at the surfaces and in the cytoplasm of the non-lymphoid cells, confirming in particular the peculiar and remarkable heterogeneity of the thymic epithelial cells not only for their ultrastructure and function, but also for their glycoconjugate content. In fact, subcapsular, paraseptal and perivascular cells differed from other epithelial cells for the presence of sialic acid, as demonstrated by the decreased reactivity with WGA lectin and the increased reactivity with SBA after neuraminidase treatment. This increase in reactivity with SBA revealed that the oligosaccharide reactive with this lectin was in part masked by sialic acid. Moreover, despite subcapsular, paraseptal and perivascular cells being commonly considered for their ultrastructure as an unique group of cells (Milicévic and Milicévic, 1997), they differed from each other for different types of glycoconjugate sugar residues present on their surface and in the cytoplasm. In fact we have demonstrated that PNA lectin reacted only with the subcapsular cells, revealing the presence of D-galactose(ß1→3)-N-acetyl-D-galactosamine within their cytoplasm and at their surface. LTA, UEA I and Con A reacted only with subcapsular and paraseptal cells. With respect to fucosyl residues, it is to be noted that LTA reacted with α-L-fucose bound via an α-(1→6) linkage to penultimate glucosaminyl residue and/or difucosylated oligosaccharides, while UEAI reacted with the same sugar residue bound via ß1,2 linkage to penultimate D-galactose-(ß1→4)-N-acetyl-D-glucosaminyl residue (Debray et al., 1981; Schulte and Spicer, 1983; Foster et al., 1991). Subcapsular and paraseptal cells could produce chemiotactic factors (Imhof et al., 1988) and thymic hormones (for review see Gaudeker et al., 1986; Milicévic and Milicévic, 1997) that stimulated the recruitment and proliferation of the T-cell precursors in the subcapsular region. All the other cortical epithelial cells showed a strong reactivity with all the used lectins, with the exception of DBA, not only at their surface but above all in granules present in their cytoplasm, thus revealing in these cells the presence of glycoconjugates with terminal ß-N-acetyl-D-galactosamine, D-galactose(ß1→3)-N-acetyl-D-galactosamine, α-D-mannose, α-D-glucosamine and α-L-fucose. These observations are in agreement with the data reported in literature (Schuurman et al., 1985; Deman et al., 1996) which indicated a synthesis of glycoprotein in these cells (Milicévic and Milicévic,1997) such as thymic hormones and other active substances. Large epithelial cells, located at the cortico-medullary boundary, were characterized by the presence of ß-N-acetyl-D-galactosamine, D-galactose(ß1→3)-N-acetyl-D-galactosamine, α-D-mannose, α-D-glucosamine and α-L-fucose as revealed by their reactivity with SBA, PNA, ConA, WGA and UEAI. In particular α-L-fucose was revealed by UEAI and not by LTA. This fact means that α-L-fucose at these cells was bound via ß1,2 linkage to penultimate D-galactose-(ß1→4)-N-acetyl-D-glucosamine. The other medullary epithelial cells reacted with SBA, PNA, ConA and WGA, but not with UEAI.

Moreover the cell surface of the T-lymphocytes reacted, with variable intensity, with ConA (cortical and medullary T-lymphocytes) and WGA (only cortical T-lymphocytes), thus revealing the presence of α-D-mannose and α-D-glucosamine.

In CSA-treated rats, in agreement with the data reported in literature, we observed a strong reduction or absence of the thymic medulla. Concurrently we observed the loss of many glycoconjugate sugar residues

on the surface and in the cytoplasm of the epithelial cells. In fact, in comparison with the control rats, the subcapsular cells were characterized by the loss of reactivity with PNA, ConA and SBA, revealing the absence of D-galactose(ß1→3)-N-acetyl-D-galactosamine, α-D-mannose, and ß-N-acetyl-D-galactosamine. Our results are in accordance with electron-microscopic data, which demonstrate the prominent morpho-functional inactivation of thymic subcapsular epithelial cells after application of CSA (Milicévic and Milicévic, 1997). The paraseptal cells were characterized by the lack of reactivity with SBA, ConA and UEAI, revealing the absence of ß-N-acetyl-D-galactosamine, α-D-mannose and α-L-fucose bound via ß1,2 linkage to penultimate D-galactose-(ß1→)-N-acetyl-D-glucosamine residue. Subcapsular, paraseptal and perivascular cells showed furthermore the loss of sialic acid as revealed by the unchanged reactivity with SBA and WGA after neuraminidase treatment. The absence of sialic acid appeared of particular significance since it could cause incomplete and/or not efficient interactions between these epithelial cells and T-lymphocytes (Varki, 1993). Although it is difficult to formulate a hypothesis on the consequences of the loss of the other glycoconjugate sugar residues in these cells, this finding could be reasonably correlate with a difficulty in the recruitment of T-cell precursors in subcapsular region and in their proliferation due to a probable reduction in the synthesis of thymic factors.

The other cortical cells, although characterised by the same lectin reactivity observed in the control rats, displayed some morphological features in agreement with a reduced synthetic activity (cellular ipotrophy, reduction of granules, longer and thinner cellular processes). Conversely, the long star-shaped epithelial cells positioned around the residual of the medulla revealed the disappearance of the binding sites for SBA and ConA in their rare granules, indicating a loss of the sugar residues ß-N-acetyl-D-galactosamine and α-D-mannose as a consequence of a change in the glycosylation processes due to CSA treatment.

Moreover the CSA treatment determined, at the surface of the T-lymphocytes, the appearance of D-galactose(ß1→3)-N-acetyl-D-galactosamine, ß-N-acetyl-D-galactosamine and sialic acid as revealed by the reactivity with PNA and SBA lectin only after neuraminidase treatment. Our results were in keeping with data of the literature reporting changes in glycosylation of surface glycolipids and/or glycoproteins and in sialilation process during T lymphocytopoiesis (Krishna and Varki, 1997; Alvarez et al., 1999). The evidence of such a large amount of terminal and subterminal sugar residues in T-lymphocytes could suggest their possible role in blocking the activation and/or in maintaining the inactivation of T-lymphocytes, as a consequence of the CSA treatment (Schauer , 1982). In CSA-treated rats, as well as in control rats, DBA was a specific marker of the macrophages (which were also identified using acid-phosphatase on cryostatic sections of thymic fragments), thus revealing the presence of anomer α-N-acetyl-galactosamine. This specific reaction allowed to evaluate an increase in number of the macrophages in the thymus of CSA-treated rats (556±45) vs control rats (114±27). Moreover in these cells sialic acid was detected as revealed by PNA reactivity after neuraminidase treatment. The increase in number of macrophages revealed in treated rats was not consistent with the data of Milicévic and Milicévic (1998). This discrepancy could be related to the modality of administration of CSA, the duration of the treatment and the daily plasmatic concentration of this immunosuppressive drug obtained with our treatment. Nevertheless it is to be noted that thymic macrophages produce a large number of active substances that could be involved in up-and-down regulation of normal T-cells production (Delebassee and Gualde, 1988). Moreover earlier reports (Milicévic et al., 1989, 1993) documented that CSA treatment induced in the macrophages marked changes in their enzyme content, ultrastructure and immunophenotype.

Our results indicate that CSA treatment induced a sophisticated kinetic of glycoconjugate expression of the thymic cells interfering with the complex function of this organ.

Apart from any other consideration the present histochemical investigation has allowed us to show for the first time a wide distributional draw of the terminal and sub-terminal sugar residues of the glycoconjugates at the sessile and motile cells of the thymic parenchyma, both in control and in CSA-treated rats.

Moreover it is necessary to emphasise that this technique allowed us to reveal the epithelial thymic cells as a whole (cellular body, cellular processes), unlike anti-cytokeratin (Colic et al., 1989, 1990; Milicévic et al., 1992) and monoclonal antibodies (De Maagd et al., 1985), which stain only portions of these cells.

References

Alvarez G., Lascurain R., Perez A., Degand P., Montano L.FG., Martinez Cairo S. and Zenteno E. (1999). Relevance of sialoglycoconjugates in murine thymocytes during maturation and selection in the thymus. Immunol. Invest. 28, 9-18

Boyd R.L., Tucek C.L., Godfrey D.I., Izon D.J., Wilson T.J., Davidson D.J., Bean A.G.D., Ladyman H.M., Ritter M.A. and Hugo P. (1993). The thymic microenviroment. Immunol. Today 14, 445-459

Colic M., Jovanovic S., Vasiljeski M. and Dujic A. (1990). Ontogeny of the rat thymic epithelium defined by monoclonal anticytokeratin antibodies. Dev. Immunol. 1, 67-75

Colic M., Jovanovic S., Mitrovic S. and Dujic A. (1989). Immunohistochemical identification of six cytokeratin-defined subsets of the rat thymic epithelial cells. Thymus 13, 175-185

De Maagd R.A., Mackenzie W.A., Schuurman H-J., Ritter M.A., Price K.M., Broekhuizen R. and Kater L. (1985). The human thymus microenvironment: heterogeneity detected by monoclonal anti-epithelial cell antibodies. Immunology 54, 745-754

De Waal E.J., Rademakers L.H.P., Shuurman H.J., Vos J.G. and Van Loveren H. (1996). Alterations of dendritic cells in the rat thymus

without epithelial cell loss during cyclosporine treatment and recovery. Toxicology 110, 133-151.

Debray H., Decout D., Strecker G. and Montreuil G. (1981). Specificity of twelve lectins towards oligosaccharides and glycopeptides related to N-glycosylproteins. Eur. J. Biochem. 117, 41-45

Delebassee S. and Gualde N. (1988). Effect of arachinoid acid metabolites on thymocyte proliferation. Ann. Inst. Pasteur (Immunol.) 139, 383-399

Deman J., Meurs M., van Claassen E., Humblet C., Boniver J. and Defresne M.P. (1996). In vivo expression in interleukin-1ß (IL-1ß), IL-2, IL-4, IL-6, tumor necrosis factor-α and interferon-γ in fetal murine thymus. Immunology 89, 152-157.

Farr A.G. and Anderson S.K. (1985). Epithelial heterogeneity in the murine thymus: fucose-specific lectins bind medullary epithelial cells. J. Immunol. 134, 2971-2977.

Fazel A.R., Sumida H., Schulte B.A. and Thompson R.P. (1989a). Lectin histochemistry of the embryonic heart: fucose binding sites in developing rats and chicks. Am. J. Anat. 184, 76-84

Fazel A.R., Thompson R.P., Sumida H. and Schulte B.A. (1989b). Lectin histochemistry of the embryonic heart: expression of terminal and penultimate galactose residues in developing rats and chicks. Am J. Anat. 184, 85-94

Feutren G. (1993). The optimum use of cyclosporin-A in autoimmune diseases. J. Autoimmun. 5 (suppl. A), 183-195.

Foster J.D., Getchell M.L and Getchell T.V. (1991). Identification of sugar residues in secretory glycoconjugates of olfactory mucosae using lectin histochemistry. Anat. Rec. 229, 525-544

Gaudecker B., von Steinmann G.G., Hausmann M.L., Harpprecht J., Milicévic N.M. and Müller Hermelink H.K. (1986). Immunohistochemical characterization of thymic microenvironment. A light microscope and ultrastructural immunocytochemical study. Cell Tissue Res. 244, 403-412.

Gheri G., Gheri Bryk S., Sgambati E. and Gulisano M. (1993). Characterization of the glycoconjugate sugar residues in developing chick esophageal epithelium. Histol. Histopathol. 8, 351-358

Gheri G., Gheri Bryk S., Sgambati E. and Balboni G.C. (1994). The nasal respiratory epithelium in human fetus: lectin histochemistry. Acta Anat. 151, 80-87

Gheri G., Gheri Bryk S. and Sgambati E. (1997). Glycoconjugate saccharidic moieties of exocrine and endocrine pancreas in the chick embryo, newborn and adult. Biotech. Histochem. 72, 158-167

Giunta M., Favre A., Ramarli D., Grossi G.E. and Corte G. (1991). A novel integrin involved in thymocyte-thymic epithelial cell interactions. J. Exp. Med. 173, 1537-1548.

Goverman J., Brabb T., Huseby E.S. and Farr A.G. (1997). TCR regulates thymic organization: lessons for TCR-transgenic mice. Immunol. Today 18, 204-209

Hess A.D. and Colombani P.N. (1987). Cyclosporin-resistant and -sensitive T-lymphocyte subsets. Ann. Inst. Pasteur (Immunol.) 138, 606-611.

Imhof B.A., Deugnier M.A., Girault J.M., Champion S., Damis C., Itoh T. and Thiery J.P. (1988). Thymotaxin: a thymic epithelial peptide chemotactic for T-cell precursor. Proc. Natl. Acad. Sci. USA 85, 7699-7703.

Janossy G., Bofill M., Trejdosiewicz L.K. and Chilosi M. (1983). Cellular differentiation of lymphoid subpopulations and their microenvironments in the human thymus. In: The thymus, Histophysiology and Pathology. Muller-Hermelink H.K. (ed). Springer Verlag-Berlin.

Krishna M. and Varki A. (1997). 9-O-Acetylation of sialomucins: a novel marker of murine CD4 T cells that is regulated during maturation and activation. J. Exp. Med. 185, 1997-2013

Lo D. and Sprent J. (1986). Identity of cells that imprint H-2-restricted T-cell specificity in the thymus. Nature 319, 672-675

Marrack P., Mc Cormack J. and Kappler J. (1989). Presentation of antigen, foreign major histocompatibility complex proteins and self by thymus cortical epithelium. Nature 338, 503-505

Milicévic N.M. and Milicévic Z. (1998). Cyclosporin A-induced changes of the thymic microenvironment. A review of morphological studies. Histol. Histopathol. 13, 1183-1196

Milicévic N.M., Milicévic Z. and Colic M. (1989). Macrophages of the rat thymus after cyclosporine treatment: Histochemical, enzymehistochemical , immunohistochemical study. Virchows Arch. (A) 57, 237-244

Milicévic Z., Zivanovic V., Todorovic V., Colic M., Kamperdijk E.W.A., Hoefsmit E.C.M. and Milicévic N.M. (1992). Differential effects of cyclosporin application on epithelial cells of the rat thymus. Immunohistochemical study. J. Comp. Pathol. 106, 25-35

Milicévic N.M., Milicévic Z., Colic M., Kamperdijk E.W.A. and Hoefsmit E.C.M. (1993). Ultrastructural studies of macrophages of the rat thymus after cyclosporin treatment. Thymus 22, 35-44

Milicévic Z. and Milicévic N.M. (1997). Ultrastructure of different types of thymic epithelial cells in normal and cyclosporin-A-treated rats. Anat. Embryol. 196,171-183

Murata F., Tsuyama S., Suzuky S., Hamada H., Ozawa M. and Muramatzu T. (1983). Distribution of glycoconjugates in the kidney studied by use of labelled lectins. J. Histochem. Cytochem. 31(1A Suppl.), 139-144

Schauer R. (1982). Chemistry, metabolism and biological functions of sialic acids. Adv. Carbohyd. Chem. Biochem. 40, 131-234.

Schulte B.A. and Spicer S.S. (1983). Histochemical evaluation of mouse and rat kidneys with lectin horseradish peroxidase conjugates. Am. J. Anat. 168, 345-362

Schuurman H.J., Wijngaert F.P., van de Delvoye L., Broekhuiren R., Mc Clure J.E., Goldstein A.L. and Kater L. (1985). Heterogeneity and age dependency of human thymus reticulo-epithelium in production of thymosin components. Thymus 7, 13-23.

Surh C.D., Ernst B. and Sprent J. (1992). Growth of epithelial cells in the thymic medulla is under control of mature T cells. J. Exp. Med. 176, 611-616

Takata K. and Hirano H. (1983). Changes in soybean agglutinin (SBA). and peanut agglutinin (PNA). binding pattern in the epidermis of developing chick embryo. Dev. Growth Diff. 25, 299-305

Van Ewijk W., Shores E.W. and Singer A. (1994). Crosstalk in the mouse thymus. Immunol. Today 15, 214-217

Varki A. (1993). Biological roles of the oligosaccharides: all the theories are correct. Glycobiology 3, 97-130

Wassef R., Cohen Z. and Langer B. (1985). Pharmacokinetic profiles of cyclosporine in rats: influence of route of administration and dosage. Transplantation 40, 489-493

Weil C. (1984). Cyclosporin-A: review of results in organ and bone-marrow transplantation in man. Med. Res. Rev. 6, 221-265.

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