Lectin-Binding Patterns in the Spermatogenic Cells of the Shiba Goat Testis

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ABSTRACT. Lectin-binding patterns in the testis of the sexually mature goat were investigated by light and transmission electron microscopy. *Dolichos biflorus* agglutinin (DBA) and *Griffonia simplicifolia* agglutinin-I (GS-I) were negative in the seminiferous epithelium, but soybean (*Glycine max*) agglutinin (SBA), *Griffonia simplicifolia* agglutinin-II (GS-II) and peanut (*Arachis hypogaea*) agglutinin (PNA) were positive in the acrosomal vesicle of the Golgi-phase spermatids and in the acrosome of the cap-, acrosome- and maturation-phase spermatids. In addition, PNA was positive also in the plasma membrane and cytoplasm of the spermatogenic cells from the late pachytene spermatocytes to the maturation-phase spermatids. This finding indicates that glycoconjugates containing D-galactose residue appear at this period. Therefore, PNA may be a useful marker of developing spermatogenic cells. Electron microscopically, the positive reactions of SBA, GS-II and PNA were demonstrated in the central dense area (acrosomal granule) of the cap-phase spermatid acrosome, but not in the peripheral low-dense area. These results indicate that the lectin-bindings in the goat testis not only are common features, but also have patterns somewhat different from those in other mammals in such a way that GS-II can be detected in the acrosomal region of all phases of spermatids and that PNA is positive in the late spermatid acrosome.—**KEY WORDS:** acrosome, glycoconjugate, goat, lectin, spermatogenic cell.

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During the last decade, a number of lectins have been employed as histochemical reagents to investigate the distribution of glycoconjugates in various tissues. Several studies have so far been made on lectin-binding patterns in the testes with particular attention to human [5–7, 10] and rodents [1, 3, 4, 8, 9, 11]. In other kinds of mammals, there are very few studies of glycoconjugates of spermatogenic cells, and, therefore, further information is required.

To our knowledge, only one report concerning the lectin-binding sites in the testes of normal adult animals at the electron microscopic level is available at present [11]. In order to define the localization of glycoconjugates in detail, it is necessary to observe the lectin-binding sites in the testes by transmission electron microscopy. The present work was conducted to study the distribution of glycoconjugates in the goat testis by light and transmission electron microscopy in comparison with other previous reports.

MATERIALS AND METHODS

Four sexually mature shiba goats were used in the present study. Under ether anesthesia, their testes were surgically excised. After washing briefly with Ringer's solution, the testes were perfused through the testicular artery with 5% glutaraldehyde in 0.05

M cacodylate buffer. The specimens were cut into smaller pieces and immersed in the same fixative for 2–3 hr.

Lectins used were soybean (*Glycine max*) agglutinin (SBA), peanut (*Arachis hypogaea*) agglutinin (PNA), *Dolichos biflorus* agglutinin (DBA), *Griffonia simplicifolia* agglutinin-I (GS-I) and *Griffonia simplicifolia* agglutinin-II (GS-II).

Staining with lectins for light microscopy: After fixation, the samples were dehydrated in a graded series of ethanol and embedded in paraffin. The sections were cut at 4 μ m in thickness, deparaffinized and rehydrated. They were treated with 1% bovine serum albumin (BSA) in 10 mM phosphate-buffered saline (PBS), pH 7.2, and incubated with biotinyl lectins (Vector Laboratory, Burlingame, CA, U.S.A., 25 μ g/ml) in 0.1% BSA-PBS for 30 min. After washing with PBS, the sections were incubated with avidin-biotinyl peroxidase complex (ABC, Vector Laboratory) for 30 min. They were then washed again with PBS, immersed in 3,3'-diaminobenzidine (DAB, 0.2 mg/ml)-H₂O₂ (0.005%) for 10 min, rinsed in distilled water, dehydrated and stained with hematoxylin.

Staining with lectins for transmission electron microscopy: After fixation, the specimens were washed with PBS for 2 hr, dehydrated in dimethylformamide and embedded in Lowicryl K4M by exposure to UV irradiation at –20°C for 1 hr. Thin

Fig. 1. Lectin-binding sites in the goat seminiferous epithelium at light microscopic levels.

- a) SBA is positive only in the acrosomal region of round spermatids, indicating the distribution of N-acetyl-D-galactosamine. $\times 300$.
- a') SBA reactions in the seminiferous epithelium at a higher magnification. arrow; SBA reaction in a late cap-phase spermatid acrosome. $\times 800$.
- b) GS-II is positive in the acrosomal region of round spermatids, in the postnuclear region of elongated spermatids and in the residual cytoplasm, indicating the existence of D-galactose. $\times 300$.
- b') GS-II reactions in the seminiferous epithelium at a higher magnification. arrow; PNA reaction in an early cap-phase spermatid acrosome. $\times 800$.
- c) PNA, representing the appearance of N-acetyl-D-glucosamine, is strongly positive in the acrosomal region of round spermatids. The plasma membrane and cytoplasm of spermatids are also reacted. $\times 300$.
- c') PNA reactions in the seminiferous epithelium at a higher magnification. $\times 800$.

sections were cut, mounted on nickel grids, treated with 1% BSA-PBS for 10 min and incubated with biotinyl lectins (12.5–25 $\mu\text{g/ml}$) in 0.1% BSA-PBS for 1 hr. After washing briefly with PBS, they were incubated with streptavidin-gold (10 nm in diameter) conjugates in 0.1% BSA-PBS for 30 min, washed again with PBS, stained with uranyl acetate and lead citrate and examined in a JEM-1200EX transmission electron microscope at 80 kV.

Negative controls (without lectins) ran in parallel in both treatments.

Ultrastructural observation: Some of the samples were washed in cacodylate buffer and postfixed in 1% osmium tetroxide/1.5% potassium ferrocyanide. They were dehydrated in graded ethanol, infiltrated in propylene oxide and embedded in Araldite. Thin sections were cut, stained with uranyl acetate and lead citrate and observed with a JEM-1200EX

transmission electron microscope at 80 kV.

RESULTS

Light microscopic observations: The spermatids in the goat seminiferous epithelium were easily subdivided into four phases (Golgi-, cap-, acrosome- and maturation-phases) by their morphological features.

GS-I and DBA exhibited no reaction in the seminiferous tubules, although GS-I was weakly positive in the endothelial cells in the interstitial region. SBA was intensely positive in the acrosomal vesicle of the Golgi-phase spermatids and in the acrosome of the cap-phase spermatids. The acrosome of the acrosome- and maturation-phase spermatids had no obvious staining with SBA (Figs. 1a, 1a'). The GS-II reaction showed a granular distribution within the seminiferous epithelium. GS-II was positive in the acrosomal region of the early round spermatids, in the postnuclear region of the maturation-phase spermatids and in the residual cytoplasm (Figs. 1b, 1b'). PNA was strongly positive in the acrosomal vesicle of the Golgi-phase spermatids and in the acrosome of the cap-, acrosome- and maturation-

Fig. 2. Electron micrograph showing two maturation-phase spermatids with well-developed acrosomes. Residual cytoplasm is also recognizable. arrows; acrosome, S; Sertoli cell, RS; residual cytoplasm. Embedded in Araldite. $\times 12,000$.

Fig. 3. Electron micrograph showing SBA-binding site in the acrosome of a cap-phase spermatid. Gold particles are distributed in the central dense area (CDA), but not in the peripheral low-dense area (*). Embedded in Lowicryl K4M. $\times 50,000$.

Fig. 4. Electron micrograph showing SBA-binding site in the acrosomes of maturation-phase spermatids. Gold particles are localized only in the acrosomes. The acrosomes are equally condensed at this period. Nu; nucleus. Embedded in Lowicryl K4M. $\times 30,000$.

Figs. 5 and 6. Electron micrographs showing GS-II (Fig. 5)- and PNA (Fig. 6)-binding sites in the acrosomes. Gold particles are found in the acrosomes of maturation-phase spermatids. Nu; nucleus, S; Sertoli cell. Embedded in Lowicryl K4M. $\times 80,000$ (Fig. 5), $\times 60,000$ (Fig. 6).

tion-phase spermatids. In addition to the acrosome, the plasma membrane and cytoplasm of all phases of spermatids were also stained with PNA. However, Sertoli cells, spermatogonia and spermatocytes had no reaction of PNA, except for a weak reaction in the cytoplasm of spermatocytes after the late pachytene stage (Figs. 1c, 1c').

Ultrastructural observations: The SBA reaction was detected not only in the acrosomal region of the Golgi- and cap-phase spermatids, but also in the acrosome of the acrosome- and maturation-phase spermatids (Figs. 3, 4). The acrosomal region of the cap-phase spermatids was composed of the centrally-located dense area (acrosomal granule) and the peripheral low-dense area. SBA was positive in the central dense area, but negative in the peripheral low-dense area (Fig. 3).

In addition to the positive reaction in the acrosomal region of Golgi- and cap-phase spermatids, GS-II was positive in the acrosome of the acrosome- and maturation-phase spermatids (Fig. 5). Moreover, in the acrosome-phase spermatids, the positive region was restricted to the central dense area of the acrosome. These findings are identical to the SBA reaction. The dense areas of the postnuclear region and residual cytoplasm were also stained with GS-II.

Similar to the SBA and GS-II reactions, PNA was detectable in the acrosomal regions of all phases of spermatids (Fig. 6). The PNA reaction was not obvious in the plasma membrane electron microscopically. Although PNA was detectable in the cytoplasm of the spermatids, its precise cytoplasmic location was not defined.

No reaction was observed in control sections.

DISCUSSION

In the present study, the acrosomal region of all phases of spermatids showed a reaction with SBA, GS-II and PNA at the electron microscopic level, indicating the distribution of N-acetyl-D-galactosamine, N-acetyl-D-glucosamine and D-galactose residues, respectively.

The DBA reaction, representing the appearance of N-acetyl-D-galactosamine residue, is controversial in the literature. Although the positive reaction of DBA is detected in the acrosomal region of the rat [1], bull [2], guinea pig [3, 11], gerbil [3], mouse [3] and nutria [3], no reaction is observed in the acrosomal region of the rat [8] and human [6]. The present study coincides with the latter finding.

Similar to the finding of the DBA reaction, the

SBA-binding patterns are slightly different among species and/or among investigators. SBA shows a reaction in the acrosome of the rat [1], bull [2], guinea pig [3, 11], gerbil [3], mouse [3], nutria [3] and human [5–7], but does not in the acrosome of the mouse [4] and rat [7]. The present finding of SBA, a positive reaction in the acrosome of early and late spermatids, is quite similar to the finding of the bull testis [2].

GS-I and GS-II were not elucidated by many authors. They reacted only with spermatozoa in the human [5] and with spermatozoa and/or spermatoocytes in the mouse [4]. In the present study, GS-I, an indicator of the distribution of D-galactose residue, showed no reaction in the seminiferous epithelium. GS-II reacted with the acrosomal region of all phases of spermatids at the electron microscopic level.

PNA has been known to show a reaction in the acrosome of the early spermatids [1, 2, 10, 11], while in the present study, it was detected in the acrosome of both the early and the late spermatids. The spermatogenic cells after the late pachytene spermatocytes demonstrated a positive reaction with PNA in their cytoplasm at the light microscopic level. This fact indicates that glycoconjugates containing D-galactose residue are newly formed within the spermatogenic cells in this period. Therefore, PNA seems to be a useful marker of developing spermatogenic cells.

The reactions of SBA, GS-II and PNA in the late spermatid acrosome were detected only at the electron microscopic level. In the acrosome of the cap-phase spermatids, the reactions were positive in the central dense region, but were negative in the peripheral low-dense region. These findings offer a confirmative support for the notion that electron microscopic studies are quite necessary to define the precise localization of glycoconjugates in the testis.

As mentioned above, the present study on the goat testis showed lectin-binding patterns similar to those of other mammals in the previous reports, but revealed features somewhat different from those in the previous reports in such a way that GS-II was

detected in the acrosomal region of all phases of spermatids and that PNA was positive in the late spermatid acrosome. Further studies are required to confirm whether these features are species-specific or not.

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REFERENCES

1. Arya, M. and Vanha-Perttula, T. 1984. Distribution of lectin binding in rat testis and epididymis. *Andrologia* 16: 495–508.
2. Arya, M. and Vanha-Perttula, T. 1985. Lectin-binding pattern of bull testis. *J. Androl.* 6: 230–242.
3. Arya, M. and Vanha-Perttula, T. 1986. Comparison of lectin-staining pattern in testis and epididymis of gerbil, guinea pig, mouse and nutria. *Am. J. Anat.* 175: 449–469.
4. Lee, M.-C. and Damjanov, I. 1984. Anatomic distribution of lectin-binding sites in mouse testis and epididymis. *Differentiation* 27: 74–81.
5. Lee, M.-C. and Damjanov, I. 1985. Lectin binding sites on human sperm and spermatogenic cells. *Anat. Rec.* 212: 282–287.
6. Malmi, R., Kallajoki, M., and Suominen, J. 1987. Distribution of glycoconjugates in human testis. A histochemical study using fluorescein- and rhodamine-conjugated lectins. *Andrologia* 19: 322–332.
7. Malmi, R. and Söderström, K.-O. 1987. Lectin binding sites in human seminiferous epithelium, in CIS cells and in seminomas. *Int. J. Androl.* 10: 157–162.
8. Malmi, R., Söderström, K.-O., and Karjalainen, K. 1983. Labeling of rat spermatogenic cells with fluorescein isothiocyanate conjugated lectins. pp. 327–333. In: *Lectins-Biology, Biochemistry, Clinical Biochemistry*, vol. 3 (Bog-Hansen, T. C. and Spengler, G. A. eds.), Walter de Gruyter and Co., Berlin, New York.
9. Söderström, K.-O., Malmi, R., and Karjalainen, K. 1984. Binding of fluorescein isothiocyanate conjugated lectins to rat spermatogenic cells in tissue sections. *Histochemistry* 80: 475–479.
10. Wollina, V., Schreiber, G., Zollmann, C., Hipler, C., and Gunther, E. 1989. Lectin-binding sites in normal human testis. *Andrologia* 21: 127–130.
11. Yamamoto, N. 1982. Electron microscopic analysis of sugar residues of glycoproteins in the acrosomes of spermatids using various lectins. *Acta Histochem. Cytochem.* 15: 139–150.