

—Original—

Lectin-Binding Patterns in the Testes of the Java Fruit Bat (*Pteropus vampyrus*) and the Japanese Lesser Horseshoe Bat (*Rhinolophus cornutus*)

Takashi MORIGAKI¹, Masamichi KUROHMARU¹, Yoshiakira KANAI¹, Mitsuru MUKOHYAMA², Eiichi HONDO³, Junzo YAMADA³, Srihadi AGUNGPRIYONO⁴ and Yoshihiro HAYASHI¹

¹Department of Veterinary Anatomy, The University of Tokyo, Tokyo 113-8657,

²Sannohe High School, Aomori 039-0141, ³Department of Veterinary Anatomy,

Obihiro University of Agriculture and Veterinary Medicine, Hokkaido 080-8535, Japan and

⁴Department of Veterinary Anatomy, Bogor Agricultural University, Bogor 16151, Indonesia

Abstract. Lectin binding patterns in the testes of the Java fruit bat, *Pteropus vampyrus*, and the Japanese lesser horseshoe bat, *Rhinolophus cornutus*, were investigated by light microscopy. Binding patterns were similar in both species, except for some slight differences. UEA-I, SBA, DBA and BSL-I revealed no reaction in the testes of either species. Con A and WGA gave a diffuse reaction all over the seminiferous epithelium in both species. In addition to this binding pattern, PHA-E exhibited a granular reaction within the cytoplasm of pachytene spermatocytes. PNA and RCA-I gave an intense reaction in the acrosomal region from Golgi to acrosome-phase spermatids in both species, but, in addition to this binding pattern, RCA-I reacted in the cytoplasm of spermatocytes and spermatids in the Java fruit bats. PSA revealed a granular reaction in the cytoplasm of spermatids in the Japanese lesser horseshoe bats. These binding patterns were similar to those of mammals studied before, except that a few specific bindings were detected in the bats.

Key words: Lectin, Japanese lesser horseshoe bat, Java fruit bat, Spermatid, Testis.

(J. Reprod. Dev. 46: 309–314, 2000)

Since lectins have a specific binding affinity for sugar residues, they have been used as histochemical reagents to investigate the distribution of glycoconjugates in various tissues. Until now, several studies have been done on lectin-binding patterns in the testes of mammalian species, such as the man [1–4], rodents [5–9], insectivore [10], common tree shrew [11], bull [12] and goat [13], but, there are no reports on the Order Chiroptera. In this study, two kinds of bats were selected: one (Java fruit bat) belongs to the

Suborder Megachiroptera, and the other (Japanese lesser horseshoe bat) belongs to the Suborder Microchiroptera. The lectin-binding patterns in the testes of these two species were investigated by light microscopy. To detect the specificity in both species, the lectin binding patterns were compared with each other and with those of other mammalian species.

Materials and Methods

Animals

Two adult Java fruit bats obtained in East Java,

Indonesia and three adult Japanese lesser horseshoe bats captured in Aomori prefecture, Japan were used in the present study. All of these bats showed signs of active spermatogenesis according to the histological analysis described below. Body weight and forearm length information are shown in Table 1.

Light microscopy

Under pentobarbital anesthesia, the animals were perfused with 0.9% physiological saline followed by Bouin's fixative through the left ventricle. The testes were excised, sliced into slabs and immersed in the same fixative overnight. They were dehydrated in a graded series of ethanol, infiltrated in xylene and embedded in paraffin wax. Sections (4 μm) were deparaffinized, stained with periodic acid-Schiff (PAS)-hematoxylin and examined by light microscopy to confirm active spermatogenesis in these animals.

Lectin histochemistry

The following lectins were used in the present study: *Ulex europaeus* agglutinin I (UEA-I), soybean (*Glycine max*) agglutinin (SBA), peanut (*Arachis hypogaea*) agglutinin (PNA), *Ricinus communis* agglutinin I (RCA-I), *Dolichos biflorus* agglutinin (DBA), *Phaseolus vulgaris* agglutinin (PHA-E), *Bandeiraea simplicifolia* I (BSL-I), Concanavalin (*Canavalia ensiformis*) agglutinin (Con A), *Pisum sativum* agglutinin (PSA) and wheat germ (*Triticum vulgare*) agglutinin (WGA).

The sections (4 μm) obtained from the paraffin block described above were deparaffinized, rehydrated and treated with 0.01 M phosphate-buffered saline (PBS) and 1% bovine serum albumin (BSA) -PBS. The sections were incubated with biotinylated lectins (Vector stain, CA, USA, 25 $\mu\text{g}/\text{ml}$) in 1% BSA-PBS for 60 min. After washing with PBS, they were treated with 1% BSA-PBS and incubated with avidin-biotin peroxidase complex (ABC, Vector stain, CA, USA) for 30 min. They were then washed again with PBS, immersed in diaminobenzidine (DAB, 0.2 mg/ml)-H₂O₂ (0.005%) for 5 min and rinsed in distilled water. They were stained with hematoxylin and observed by light microscopy.

Negative controls without lectins were processed in parallel.

Table 1. The body weight and forearm length of the Java fruit bat and the Japanese lesser horseshoe bat

Species	weight (g)	forearm length (mm)
Java fruit bat ①	613	175
Java fruit bat ②	521	175
Japanese lesser horseshoe bat ①	7.2	41.0
Japanese lesser horseshoe bat ②	6.8	41.4
Japanese lesser horseshoe bat ③	6.9	41.4

Results

Java fruit bat

The spermatids of the Java fruit bats could be easily subdivided into 4 phases (Golgi, cap, acrosome and maturation-phases). Six lectins (PNA, PHA-E, Con A, WGA, RCA-I and PSA) exhibited a positive reaction in spermatogenic cells, whereas four lectins (UEA-I, SBA, DBA and BSL-I) revealed a negative reaction in the testes. PNA and RCA-I showed an intense reaction in the acrosomal region of spermatids. In short, PNA was intensely positive in the acrosomal region from Golgi to acrosome-phase spermatids (Fig. 1a). This reaction disappeared in maturation-phase spermatids. RCA-I gave an intense reaction in the acrosomal region from Golgi to acrosome-phase spermatids and also reacted in the cytoplasm of spermatocytes and spermatids (Fig. 1b). In addition to a diffuse reaction in the seminiferous epithelium, PHA-E exhibited a granular reaction within the cytoplasm of pachytene spermatocytes (Fig. 1c). Although Con A and WGA revealed a diffuse reaction all over the seminiferous epithelium, WGA showed an intense reaction in the cytoplasm of acrosome-phase spermatids and Sertoli cells (Fig. 1d). PSA showed a weak reaction in the interstitial region, Sertoli cells and elongate spermatids.

Japanese lesser horseshoe bat

The spermatids of the Japanese lesser horseshoe bats could also be subdivided into 4 phases (Golgi, cap, acrosome and maturation-phases). In most cases, the lectin binding patterns in the testes of the Japanese lesser horseshoe bats were similar to those of the Java fruit bats (Fig. 2a, b), but they were different in the RCA-I and PSA reactions. In the Japanese horseshoe bats, RCA-I was strongly positive in the acrosomal region from Golgi to acrosome-phase spermatids as well as in the Java

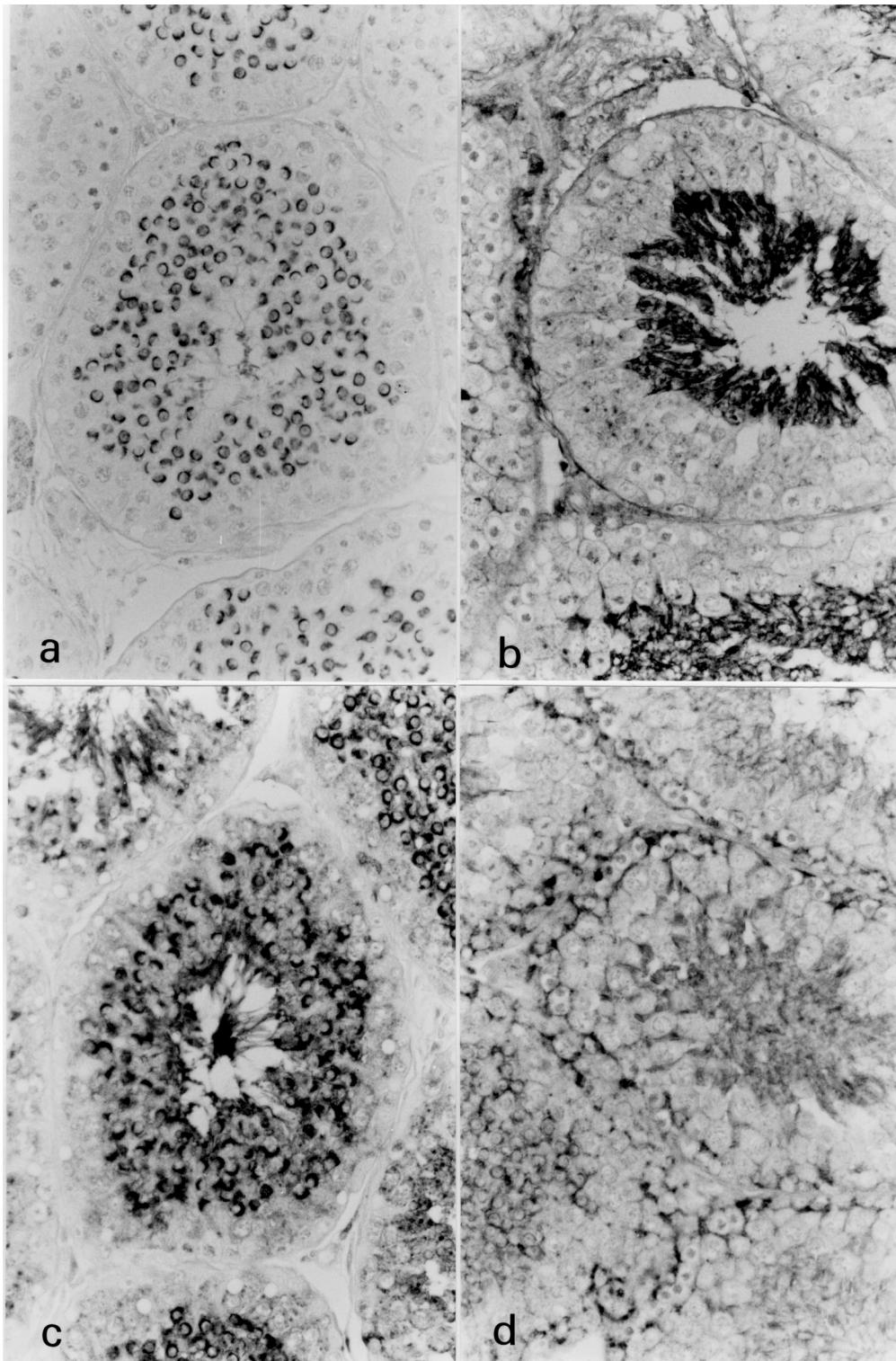


Fig. 1. Lectin binding sites in the testis of the Japanese lesser horseshoe bat. (a) PNA is strongly positive in the acrosomal region. (b) PHA-E exhibits a granular reaction within the cytoplasm of pachytene spermatocytes in addition to a diffuse reaction in the seminiferous epithelium. (c) RCA-I reacts strongly in the acrosomal region and reacts in the cytoplasm of spermatocytes and spermatids. (d) WGA reveals a diffuse reaction all over the seminiferous epithelium and reacts strongly in the cytoplasm of acrosome-phase spermatids and Sertoli cells. $\times 320$.