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Immunocytochemical alterations in the intra-acrosomal antigen MN7 during epididymal maturation of guinea pig spermatozoa

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Abstract We have previously shown that a 90-kDa intra-acrosomal antigen, MN7, is restricted to the anterior acrosomal region of mouse, rat, and hamster spermatozoa. The present study has examined the localization and the behavior of MN7 during sperm maturation in the epididymis of the guinea pig by immunoelectron microscopy. MN7 showed not only a specific localization in the apical segment of the guinea pig sperm acrosome, but also a distinct alteration during maturation, as follows. MN7 was exclusively found both at the dorsal matrix and on the outer acrosome membrane (OAM)/matrix-associated materials in the apical segment. MN7 was initially distributed throughout the electron-lucent dorsal matrix in immature sperm but, during maturation, became more restricted to the spherical bodies within the electron-lucent area. MN7 on OAM/matrix-associated materials was first distributed along the ventral margin and the small area posterior to the dorsal matrix but, during maturation, disappeared from the ventral margin and became restricted to the dorsal region. These results indicate that MN7 is a good tool for studying the stepwise maturation of epididymal spermatozoa.

Key words Acrosome · Epididymal maturation · Monoclonal antibody · Immunocytochemistry · Spermatozoa · Guinea pig

Introduction

After leaving the testis, mammalian spermatozoa continue to undergo a series of morphological, physiological, and biochemical alterations as they pass through the epididy-
Materials and methods

Animals

Adult male guinea pigs (Hartley strain) weighing 600–700 g were purchased from Kyudo (Kumanoto, Japan). Animals were maintained in the Laboratory Animal Center of Miyazaki Medical College in an automatically controlled environment (12 h light/12 h dark, 20°C) with free access to food and water under the guideline for animal welfare of our college. They were anesthetized with ether and whole epididymides were removed.

Monoclonal antibody

The monoclonal antibody against MN7 (mMN7, IgG1) used in this study was produced in BALB/c female mice immunized with washed CD-1 cauda epididymal spermatozoa (Toshimori et al. 1990). It has been characterized by Tanii et al. (1994).

Immunohistochemistry at the light-microscopic level

Fully mature spermatozoa flushed from the distal part of the cauda epididymal duct were washed with phosphate-buffered saline (PBS, pH 7.4) by centrifugation at 90g for 5 min. The spermatozoa were then fixed on ice with 1% paraformaldehyde for 20 min and attached to silane-coated glass slides. Immunostaining for light microscopy was carried out at room temperature as follows. Specimens were blocked with 5% normal goat serum and incubated with mMN7 for 30 min. They were then washed with PBS and incubated with biotinylated goat anti-mouse IgG (DAKO, Carpenteria, Calif.), followed by streptavidin and biotin-peroxidase complex (DAKO). After being washed with PBS, the immunocomplexes were visualized with 0.05% diaminobenzidine (DAB) and observed without counterstaining. Control experiments were carried out with normal mouse IgG instead of mMN7. Non-specific staining was also checked by omitting the incubation step with the first antibody.

Immunoelectron microscopy

Each epididymis was dissected into several segments as depicted in Fig. 1, according to the morphological criteria of Hoffer and Greenberg (1978). The epididymal segments were fixed with periodate-lysine-2% paraformaldehyde (PLP) fixative for at least 4 h at 4°C. They were sequentially washed with PBS containing sucrose and quickly frozen in OCT compound (Miles, Elkhart, Ind.). Frozen sections were cut (6 μm thick) in a Leitz 1720 cryostat (Wetzlar, Germany), blocked with 5% normal goat serum, and incubated with mMN7 at 4°C overnight. After being washed with PBS, the sections were incubated with the horseradish-peroxidase-conjugated Fab’ fragment of goat anti-mouse IgG (Protos Immunoresearch, San Francisco, Calif.), fixed with 0.5% glutaraldehyde, reacted with 0.05% DAB, and post-fixed with 2% OsO4. The sections were dehydrated in ethanol and embedded in Epon 812. Ultrathin sections were examined without counterstaining under a Hitachi H-7100 transmission electron microscope (Tokyo, Japan) operated at 75 kV. Control experiments were carried out with normal mouse IgG instead of mMN7. Non-specific staining was also checked by omitting the incubation step with the first antibody.

Conventional transmission electron microscopy

The various segments of the epididymis were cut into small blocks and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer for at least 2 h at 4°C. After being rinsed in the same buffer, the samples were post-fixed for 1 h with 2% OsO4 in the buffer, dehydrated through graded ethanol, and routinely embedded in Epon. Ultrathin sections were counterstained with uranyl acetate and lead citrate and then examined under the transmission electron microscope operated at 75 kV.

Scanning electron microscopy

The distal part of the cauda epididymis filled with fully mature spermatozoa was fixed in the same way as for the conventional transmission electron microscopy. After being rinsed in the buffer, epididymal tubules were transversely or longitudinally cut with razor blades into 1-mm-thick slices. They were dehydrated through graded ethanol, dried by the freeze-drying method with t-butyl alcohol (Eiko ID-2, Tokyo, Japan), sputter-coated with palladium-gold, and then observed under a Hitachi S-800 scanning electron microscope operated at 15 kV.

Results

Light-microscopic level

A positive reaction to mMN7 was found in the acrosomal region (anterior acrosome) of mature sperm of the guinea pig. The reaction was intense at the apical segment extending beyond the anterior margin of the nucleus, whereas the principal segment covering the anterior part of the nucleus showed weak or very little reaction (Fig. 2). In the apical segment, intense reaction was detected at the inner region of the dorsal aspect forming horseshoe-shaped bands, but the reactivity tended to decrease gradually at the anterior margin (Fig. 2). Scanning electron microscopy revealed that such mMN7-positive bands corresponded to the posterior sites of the dorsal bulge of the apical segment (Fig. 3). No reaction was observed in the equatorial segment, postacrosomal region, or the flagellum. The control specimens showed no significant reaction (data not shown).

Electron-microscopic level

As shown in Figs. 4 and 5, the characteristic morphology of the acrosome of the fully mature sperm of the guinea pig was not found in spermatozoa in zone I (which corresponds to the caput and corpus epididymidis in other species) or zone II (proximal cauda) of the epididymis but was gradually acquired during the passage of the spermatozoa through zone III (proximal cauda) and subsequent zones (medial and distal cauda; Fig. 1). This finding is consistent with those of previous electron-microscopic studies (Fawcett and Hollenberg 1963; Holt 1979).

In zones I or II, the apical segment of the immature sperm acrosome showed three matrix zones that could be distinguished by a very slight difference in their electron density, viz., an electron-lucent dorsal matrix, an intermediate matrix, and an electron-dense ventral matrix (Fig. 4a). Immunelectron microscopy revealed that MN7 was broadly distributed within the dorsal matrix, the domain designated M1 by Olson et al. (1987); the reactivity was more intense at the antero-medial side than at the postero-lateral sides (Fig. 4b–d). MN7 was also localized to the outer acrosomal membrane (OAM)/matrix-associated materials, both along the ventral margin and at the dorsal margin of the apical segment, whereas the plasma membrane covering the sperm did not react to mMN7.