Ultrastructural Aspects of Spermatids in Isogenic Black Mouse C57BL6J

Aspectos Ultraestructurales de las Espermátidas en el Ratón Negro Isogénico C57BL6

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SUMMARY: The objective of this study was to describe some changes in spermatids differentiating events during spermiogenesis, leading to spermatozoa final formation. The animals were sacrificed with ethylic acid inhalation and, then, testis fragments were collected, fixed in Karnovsky and post-fixed in osmium tetroxide. After dehydration, the sections were stained with uranyl acetate 2%. It was observed in early spermatids at Golgi phase, formation of acrosomal granule contained in the acrosomal vesicle, spheroidal nucleus with rugous reticulum around it, and Golgi apparatus made up of lamellae and vesicular structures. At cephalic hood phase, the granule suffers flattening and the marginal fossa is formed. In both phases, it was possible to observe the presence of multivesicular body. At acrosomal phase, the acrosomal system is set up, and several enzymes of endoplasmatic reticulum as well chromatoid body are observed in the cytoplasm. At the last phase, that is, maturation, the nucleus becomes more elongated, the endoplasmatic reticulum regresses, the Golgi apparatus approaches to the flagellum and mitochondrias align next to it.

KEY WORDS: Spermiogenesis; Spermatids; Mice.

INTRODUCTION

In mammalian spermiogenesis, marking nucleocytoplasmatic changes and evolutionary alterations of the cytoplasmatic organules involved in the formation of cephalic cap, acrosome, flagellum, round spermatids, elongated spermatids and spermatozoa, are ultra-structurally characterized.

It has been shown by light and electron microscopy, that the Golgi apparatus in early spermatids is the main organelle involved in acrosome formation (Burgos *et al.*, 1970; Mollenhauer *et al.*, 1976; Burgos & Gutiérrez, 1986). One of the most important features of the Golgi apparatus is that its product and wrapping membrane are initially stored in the acrosome, before being immediately secreted into the extra-cellular mean (Burgos & Gutiérrez).

The acrosome development during spermiogenesis is well documented by Leblond & Clermont, (1952); Bedford & Nicander, (1971); Plöen, (1971); Phillips, (1972); Dym, (1977), Maxwell, (1982).

Studies performed with enzymatic activities and tritiated sugars in rat spermatid report that the Golgi apparatus

in early spermatids produces, besides the pro-acrosomal granule, multivesicular body, which associates to the chromatoid body (Tang *et al.*, 1982).

The chromatoid body is transitorily associated with the Golgi apparatus in round spermatids (Fawcett *et al.*, 1970; Susi & Clermont, 1970; Parvinen & Jokelainen, 1974; Thorne-Tjomsland *et al.*, 1988). Its structures is primarily made up with filamentous granule material and is related to carbohydrate-rich vesicles (Susi & Clermont; Thorne-Tjomsland *et al.*). On the other hand, based on histochemical reactions, Sud (1961); Fawcett *et al.*, concluded that it is composed of basic proteins and ribonucleic acid. According to Fawcett *et al.*, there is no evidence to support the nuclear origin of chromatoid body, however, Comings & Okada (1972) postulates a probable nuclear origin of this structure.

Little attention has been given to another cellular component, the endoplasmatic reticulum, which also suffers structural changes during spermiogenesis. However, some studies on its development and evolution have been done during human spermiogenesis (Nistal *et al.*, 1980); in rodents (Clermont & Rambourg, 1978), and in bats (Orsi *et al.*, 1992).

MATERIAL AND METHOD

Five 50 to 60-day old, isogenic black mice C57BL6J were sacrificed with ethylic ether inhalation, and then had their testicles removed. For light microscopy analysis, one testis was fixed in Bouin and the sections stained with hematoxylin and eosin, while the other was fixed in Karnovsky's for 4 hours, post-fixed in osmium tetroxide 1% and in phosphate buffer for 2 hours, for electron microscopy. After dehydration, the testis were embedded in Araldite and the sections stained with uranyl acetate 2%. The analysis was done by using transmission electron microscopy Philips EM 301, where cellular aspects were photographed.

RESULTS

During the spermiogenesis process of isogenic black mice, it was observed in early spermatids (round) at Golgi phase, some changes in the Golgi apparatus region, which contributes with carbohydrate-rich material to form 2 or 3

pro-acrosome granules that associate to form the acrosome granule contained in an acrosomal vesicle. In this phase, the cells presented spheroidal nucleus and rugous endoplasmatic reticulum, located preferentially around the nucleus. The Golgi apparatus showed to be made up of lamellae, saculations and vesicular structures, keeping relations of adjacence with the endoplasmatic reticulum, forming a discontinuous cover around it (Fig.1).

At cephalic cap phase, the acrosomal granule flattened on the nucleus, and the cephalic cap developed in the nucleus cranial pole. The nucleus portion adjacent to the acrosomal vesicle posterior margin suffered an alteration in its contour and formed the marginal fossa (Fig.2a), causing a light enlargement in the subacrosomal space (Fig.2b). In both phases, it was possible to observe the presence of multivesicular body, a thick structure delimited by a membrane full of small vesicles loosen in its interior (Fig. 3)

At acrosomal phase (Fig.4), one-third of the nucleus was covered by the cephalic cap and acrosomal granule. The combination of acrosome and acrosomal vesicle formed the acrosomal system, usually referred to as

acrosome. At the beginning of this phase, the nucleus shape turned from spheric to ovoid, while in the cytoplasm, it was noticed large quantity of endoplasmatic reticulum vesicles and chromatoid body, which migrated slowly toward the cell caudal pole located oppositely to the acrosomal system. As these cells approached the following phase, the nucleus shape kept changing, becoming more and more elongated until maturation phase (Fig.5), where the endoplasmatic reticulum regressed, contributing actively, with its changes, to differentiate spermatids to spermatozoa. The Golgi apparatus turned to occupy a central site in the cytoplasm, near the flagellum, and the mitochondrias were aligned along the flagellum fibrous components.

Fig. 1. Spermatid at Golgi phase. Golgi apparatus (GA), acrosomal granule (AG), nucleus (N), rugous endoplasmatic reticulum (RER) and vesicular structures (VS) are observed.

Fig. 2: Spermatid at cephalic cap phase; a) external acrosomal membrane (EAM), acrosomal vesicle (AV), marginal fossa (MF); b) Golgi apparatus (GA), acrosomal vesicle (AV), nucleus (N) and subacrosomal space (arrow).

DISCUSSION

The major stages of spermatids development, which characterizes spermiogenesis, were described by several authors in different mammals, with the use of optical microscopy (Clermont, 1972). In rodents, including mouse albino (Leblond & Clermont; Oakberg, 1956) and isogenic black mouse (Martins, 1993, 1999 and Martins *et al.*, 2001), the different stages and phases of the spermatogenetic process were characterized.

During the cellular growth and secretion within the spermatids differentiating process in spermiogenesis, Burgos & Gutiérrez, refer to Golgi apparatus as a dynamic cytoplasmatic structure.

As previously established by Jamieson & Palade, 1977, Susi *et al.*, 1971 and Mollenhauer *et al.* the proteins are synthesized in the rugous endoplasmatic reticulum, transported to Golgi apparatus, where they are transformed into glycoproteins and released for the acrosomal system development. In this process context, Mollenhauer *et al.*, recognized, in guinea pig early spermatids, the existence of intermediate and condensed vesicles related with the rugous endoplasmatic reticulum and acrosome, respectively, being that the vesicles presented characteristics common to the plasmatic membrane. In our results, it could be observed in early spermatids (Golgi phase), vesicular structures next to Golgi apparatus, agreeing with Mollenhauer *et al.*, who suggest

Fig. 3: Spermatid at the initial phase of the spermiogenesis. Nucleus (N), intercellular bridge (IB), multivesicular body (MB). In set: multivesicular body (MB).

their function in the transport of reticulum-Golgi-acrosome products.

According to Sun *et al.*, 1977; Nistal *et al.* and Paniagua, 1986, these vesicular structures correspond to annular lamellae, probably originated from the nuclear envelope, which would contribute to the Golgi apparatus development and, indirectly, to acrosome formation.

Differing a little from Tang *et al.*, who highlights the presence of multi-vesicular body only at Golgi phase, our results show such structure either at Golgi phase or cephalic hood phase. On the other hand, we agree with these authors, who assume that the multi-vesicular body derives from elements of the Golgi apparatus.

As observed by Nistal *et al.*, the presence of annular lamellae at acrosomal and maturation phases in opposite position to acrosome, was also characterized

in isogenic black mouse in this study. Holstein & Roosen-Runge, 1981; Paniagua *et al.*, 1986 consider that the destiny of the chromatoid body and annular lamellae is the spermatids neck region, which also contains ribonucleoprotein (Esponda, 1983; Czaker, 1985), suggesting that the chromatoid body and annular lamellae may be involved with the transport of these proteins to structures of the spermatids neck region.

Fig. 4: Spermatid in final stage of acrosomal phase. Nucleus (N), acrosomal system (AS), acrosomal system stalks: dorsal stalk (DS) and ventral stalk (VS).

Fig. 5: Spermatid at maturation phase. Elongated nucleus (N), mitochondrias (M) aligned along the flagellum.

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RESUMEN: El objetivo del estudio, fue describir algunas alteraciones producidas en la espermiogénesis, en los eventos que diferencian a las espermátidas y que llevan a la formación final de los espermatozoides. Los ratones fueron sacrificados con inhalación de éter etílico y, enseguida, se recolectaron fragmentos de testículos, los que fueron fijados en Karnovsky y tetróxido de osmio. Después de la deshidratación, los cortes fueron teñidos con acetato de uranilo al 2 %. En espermátidas jóvenes en la fase de Golgi se observan: formación del gránulo acrosómico contenido en la vesícula acrosómica, núcleo esférico con retículo rugoso a su alrededor, y aparato de Golgi formado por lamelas y estructuras vesiculares. En la fase de capuz cefálico, el gránulo sufre achatamiento y se forma la fosa marginal. En estas dos fases fue posible observar la presencia del cuerpo multivesicular. En la fase acrosómica se establece el sistema acrosómico, distinguiéndose en el citoplasma muchas vesículas de retículo endoplasmático y cuerpo cromatoide. En la última fase, la de maduración, el núcleo se torna más alargado, el retículo endoplasmático retrae, el aparato de Golgi se aproxima al flagelo y las mitocondrias se alinean a lo largo de él.

PALABRAS CLAVE: Espermiogénesis, Espermátidas; Ratones.

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