Continual Maintenance of the Blood-Testis Barrier During Spermatogenesis: The Intermediate Compartment Theory Revisited

Futoshi YAZAMA

1)Laboratory of Cell Biology and Morphology, Department of Life Science, Prefectural University of Hiroshima, Hiroshima 727-0023, Japan

Abstract. Tight junctions occur between the lateral processes of neighboring Sertoli cells that divide the seminiferous epithelium into two compartments: basal and adluminal compartments. These tight junctions constitute the blood-testis barrier (BTB). The established theory that the BTB must open when spermatocytes translocate from the basal compartment to the adluminal compartment is marked by one contradiction, that is, normal spermatogenesis occurs in the testis because the BTB is expected to constantly seclude the adluminal compartment from the basal compartment in order to protect haploid germ cells from the autoimmune system. Subsequently, another concept was proposed in which two BTBs divide the seminiferous epithelium into three compartments: basal, intermediate and adluminal compartments. It has been suggested that the transition from the basal region to the adluminal region without the BTB open occurs through the agency of a short-lived intermediate compartment embodying some primary spermatocytes. In contrast, the results of recent findings in the molecular architecture of the BTB suggest that the BTB in the seminiferous epithelium must “open”. In this paper, I re-examine the BTBs of boar and experimental cryptorchid mouse testes by transmission electron microscope (TEM). TEM analysis showed that an atypical basal compartment existed in the thin seminiferous epithelium of 14-day post-cryptorchid mice testes. In developmental boar testes, ectoplasmic specialization (ES) of the seminiferous epithelium showed dynamic behavior. The intermediate compartment was clearly observed between the basal and adluminal compartments of the mature boar seminiferous epithelium. ESs were observed between Sertoli cells and spermatids at all developmental stages, including early, late and mature. Furthermore, ESs were situated on the apical surface of the seminiferous epithelium. From these results, I propose that the BTB is continually maintained during spermatogenesis and suggest a model of ES circulation in the seminiferous epithelium.

Key words: Blood-testis barrier, Cryptorchidism, Ectoplasmic specialization, Testis, Transmission electron microscope (TEM)

Most epithelia have tight junctions between their cells, just below their free surfaces. The seminiferous epithelium is exceptional in having tight junctions nearer the base of the epithelium. These tight junctions occur between the lateral processes of neighboring Sertoli cells that arch over the intervening spermatogonia. They divide the seminiferous epithelium into two compartments: a basal compartment containing spermatogonia and an adluminal compartment containing the later stage of germ cell differentiation. These tight junctions constitute the blood-testis barrier (BTB), and the BTB must open to accommodate elevation of the spermatocyte. This theory was established by Dym and Fawcett in 1970 [1]. The BTB is a dynamic structure that undergoes cycles of “opening” and “closing” during the epithelial cycle to facilitate germ cell migration, yet its integrity must not be compromised in order to maintain the microenvironment behind it. Another concept was subsequently proposed by Russell in 1977 [2]. He showed an intermediate compartment and described the seminiferous epithelium divided into three compartments by two BTBs. He postulated a transient intermediate compartment of the seminiferous epithelium and explained spermatocyte elevation without the BTB open for the first time, and this concept has been accepted as part of the general understanding of germ cell elevation [3].

On the other hand, recent advances concerning the molecular architecture of the BTB have shifted attention to understanding some of the key events that regulate spermatogenesis, such as opening and closing of the BTB to permit timely passage of primary spermatocytes across the it [4–8, 35–37]. The results of recent research in this area also suggest that the BTB in the seminiferous epithelium must “open” to accommodate migration of primary spermatocytes from the basal compartment to the adluminal compartment.

The BTB is composed of four elements: tight junctions, Sertoli cell membranes, microfilament bundles and subsurface cisternae of endoplasmic reticula. The last three elements of the BTB are referred to as ectoplasmic specialization (ES) [9]. The structure and function of the BTB and/or ES have been studied in various species [1–3, 9–29, 38–40]. The boar seems to be one of the best experimental animals for investigation of ES, especially because microfilaments of ES are more clearly visualized in boars than in other mammals [29, 38]. In fact, the seminiferous epithelium contains numerous cells of different types during spermatogenesis. Thus, it is not easy to identify whether the BTB opens to accommodate migration of spermatocytes from the basal compartment to the
adluminal compartment in normal testes. Although cryptorchidism severely affects spermatogenesis and results in the rapid loss of haploid germ cells [30, 31], the population of spermatogonia is not modified by experimental cryptorchidism in many species [32, 33]. The BTB also does not change morphologically, and the integrity of the BTB is not affected in experimental cryptorchidism [34]. This experimental model might be valuable for examination of whether the BTB is open.

In this study, I re-examined the BTBs of boar and experimental cryptorchid mouse testes by TEM. The morphological data obtained suggest that the BTB need not open during spermatocyte elevation from the basal compartment to the adluminal compartment via the intermediate compartment in the seminiferous epithelium, and this model also allows for continual maintenance of the BTB.

Materials and Methods

The protocol for this research project was approved by a suitably constituted Ethics Committee of the institution within which the work was undertaken and conforms to the provisions of the Declaration of Helsinki, 1995 (as revised in Edinburgh, 2000).

Animals

A total of 22 boar (Landrace breed) testes used were from five mature (n=10), two 2-month-old (2M; n=4), two 4-month-old (4M; n=4) and two 6-month-old (6M; n=4) boars. Forty-eight C57BL/6J male mice (35-days-old) were used.

Morphological analyses

Boar testes were removed and perfused with Ringer’s solution via the testicular artery for about 5 min. A perfusion fixative, composed of 2% paraformaldehyde (PFA) and 4% glutaraldehyde (GA) in 0.1 M cacodylate buffer (pH 7.2), was applied for about 20 min. at room temperature (RT).

The C57BL/6J male mice (35-day-old) were made unilaterally cryptorchid by surgery, and scrotal testes were used as control samples. Fourteen days after the cryptorcid operation, the mice (49-day-old) were fully anesthetized with pentobarbital and perfused with 2% PFA and 4% GA in 0.1 M phosphate buffer (pH 7.2) via the left ventricle at RT and then immersed in the same fixative at RT for 2 h.

For conventional TEM, small pieces of fixed testis tissue were post-fixed with 1% buffered osmium (pH 7.2) at 4 C for 90 min., dehydrated using an ethanol series and embedded in EPON 812 (TAAB Laboratories, Berkshire, England). Semithin sections stained with 1% Toluidine blue dye (TB) were examined under a light microscope (BH-2; Olympus, Tokyo, Japan). Ultrathin sections were doubly stained with uranyl acetate and lead nitrate and then examined with a transmission electron microscope (TEM) (JEM-1200EX; JEOL, Tokyo, Japan) operated at 80 kV.

As an intercellular tracer, 2% aqueous lanthanum nitrate was mixed with an equal volume of 4% GA and used to fix the testis tissue [29]. Freeze-fracture replica analysis was performed as described previously [38].

Results

ES in the mature boar testis

In the basal part of the seminiferous epithelium, the BTB was clearly identified between adjacent Sertoli cells. The electron tracer penetrated the intercellular cleft around the spermatogonium, invaded the interspace between adjacent Sertoli cells and stopped abruptly upon encountering the BTB (Fig.1A).

The intermediate compartment was clearly observed between the basal and adluminal compartments of the mature boar seminif-
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ERIOUS epithelium. The BTB was located at both above and below the spermatocyte (Fig. 1B).

Ectoplasmic specializations were observed between Sertoli cells and the early spermatid (Fig. 1C) and between Sertoli cells and the late spermatid (Fig. 1D).

Ectoplasmic specializations were also observed between Sertoli cells and mature spermatids (Fig. 1E).

Ectoplasmic specializations were situated on the apical surface of the seminiferous epithelium (Fig. 1F).

Figure 2 shows higher magnification of the electron micrographs of ES in Figs. 1C, D, E and F. This figure shows ES between Sertoli cells and the early spermatid (Fig. 2A), ES between Sertoli cells and an acrosomal phase spermatid (Fig. 2B), ES between Sertoli cells and the mature spermatid (Fig. 2C) and ES positioned on the apical surface of the seminiferous epithelium (Fig. 2D). All of these ectoplasmic specializations were composed of Sertoli cell membranes, bundles of microfilaments (MF) and subsurface cisternae of endoplasmic reticula (SC).

ES in the developmental boar testis

In the basal area of the seminiferous epithelium of 2-month-old (2M) boar testes, an ES was clearly observed to be lying against the basement membrane (BM) of the seminiferous epithelium (Fig. 3A).

In 4M boar testes, MFs and SCs were positioned away from the BM of the seminiferous epithelium (Fig. 3B).

In 6M boar testes, multiple ESs were identified between adjacent Sertoli cells in the basal area of the seminiferous epithelium (Fig. 3C).

Figure 3D shows the basal aspect of the seminiferous epithelium of mature boar testes and the junctional vesicle (JV) detected in Sertoli cells just above the spermatogonia.

BTB in the cryptorchid mouse testis

At the light microscopic level, no morphological or size differ-

Fig. 2. Higher magnifications of electron micrographs of ES in the mature boar testis. (A) ES of the interface between Sertoli cells and the early spermatid in a higher magnification of Fig. 1C (curly brace with asterisk). (B) ES of the interface between Sertoli cells and the late spermatid in a higher magnification of Fig. 1D (curly brace with asterisk). (C) ES of the interface between Sertoli cells and the mature spermatid in a higher magnification of Fig. 1E (curly brace with asterisk). (D) ES of the apical surface of the seminiferous epithelium in a higher magnification of Fig. 1F (curly brace with asterisk). MF, microfilament bundles; SC, subsurface cisternae of endoplasmic reticula; L, lumen of seminiferous tubule. Scale bar, 500 nm.

Fig. 3. ES in the developmental boar testis. (A) Basal area of the seminiferous epithelium of the 2M boar testis. (B) Basal area of the seminiferous epithelium of the 4M boar testis. (C) Basal area of the seminiferous epithelium of the 6M boar testis. (D) Basal area of the seminiferous epithelium of the mature boar testis. The ES lies against the BM of the seminiferous epithelium (A), migrates away from the BM (B, arrow) and assembles the BTB (C). Junctional vesicle (JV) observed above the spermatogonia (D). Scale bar, 200 nm. S, Sertoli cells; SPG, spermatogonium; MF, microfilament bundles; SC, subsurface cisternae of endoplasmic reticulum.
ences were detected in the seminiferous tubules between the control testes of 35- and 49-day-old mice (Figs. 4A and B). These seminiferous epithelia are relatively thick because they contain numerous cells at all stages of spermatogenesis. By contrast, degenerating seminiferous tubules were observed in 14-day post-cryptorchid mice. There was no evidence of spermiogenesis (Fig. 1C). TEM analysis showed that the thin seminiferous epithelium of 14-day post-cryptorchid mice testes was composed of spermatogonia that lay against the BM of the seminiferous epithelium, spermatocytes positioned away from the BM and Sertoli cells (Fig. 4D). The typical BTB was recognized above the spermatogonium.

Figure 5A shows the basal aspect of the seminiferous epithelium of 14-day post-cryptorchid mice testes after administration of lanthanum. The electron tracer penetrated the intercellular cleft around the spermatocyte and stopped just above the spermatocyte. At high magnification, well-defined lanthanum deposits were observed in the intercellular spaces. Lanthanum penetrated a short distance into the inter-Sertoli cell junctions, giving the gap a striated appearance.
Discussion

Germ cell movement across the seminiferous epithelium during spermatogenesis is associated with extensive junction restructuring [4–8], including dynamic changes in the BTB [2, 9, 10, 35–37]. As shown in Figs. 1 and 2, ESs were observed between Sertoli cells and spermatids from the early to mature stages and were also situated on the apical surface of the seminiferous epithelium in the mature boar testis. In developmental boar testes, ES near the base of the seminiferous epithelium showed dynamic behavior. ES lay against the BM of the seminiferous epithelium in 2M (Fig. 3A) and away from the BM of the seminiferous epithelium in 4M (Fig. 3B), and multiple ESs were assembled at the base of the seminiferous epithelium in 6M, which is the interface between Sertoli cells (Fig.
Because the BTB is constructed with two ES units in the TEM image [9], it is reasonable to assume that the ESs associated with early, late and mature spermatids and the apical surface of the seminiferous epithelium are remnants of BTBs after they dissociate to open the junction. The junctional vesicle was also observed in mature boar testes (Fig. 3D; JV). Junctional vesicles as endocytosed junctions may be transported from the apical to basal area of the seminiferous epithelium and may be recycled for use in development of a new BTB between spermatogonia and spermatocytes [39].

In agreement with a previous study [34], TEM analysis showed that the BTB does not change morphologically and that the integrity of the BTB is not affected by experimental cryptorchidism (Figs. 4D and 5). Because the BTB was not observed in some cases between the BM and spermatocytes after they left the BM (Fig. 5A), formation of the BTB is not strictly coupled with departure of spermatocytes from the BM. There may be other cues that trigger formation of a BTB. The thin seminiferous epithelia of 14-day post-cryptorchid mice testes are composed of spermatogonia, spermatocytes and Sertoli cells (Fig. 4D), and these cells are diploid. In many species, the population of spermatogonia is unaffected by experimental cryptorchidism, allowing new spermatogenetic cycles to start again if a normal temperature is restored within a reasonable period of time [32, 33]. Therefore, the loss of haploid germ cells in cryptorchid testes may be due to meiosis arrest.

Figure 6 shows a schematic drawing illustrating the dynamics of ES during spermatogenesis in the seminiferous epithelium. The typical BTB is situated above the spermatogonia (Fig. 6A; see Figs. 1A and 4D), and this localization of the BTB and compartmentation of the seminiferous epithelium correspond to the established theory [1]. Entry of tracers into the seminiferous epithelium is prevented by the BTB (Fig. 1A; arrow). In the atypical basal compartment, where the BTB is seen above the spermatocyte (Fig. 6B), tracers consequently are able to surround the spermatocyte, and the BTB above the germ cell prevents further passage of tracers into the seminiferous epithelium (Fig. 5A; arrows). In turn, the new BTB is assembled below the spermatocyte, and the intermediate compartment is organized at the basal area of the seminiferous epithelium (Fig. 6C; see Fig. 1B). It has also been reported that an intermediate compartment could be located between the basal and adluminal compartments of the rat testis. This suggestion of an intermediate compartment would explain the passage of spermatocytes from the basal compartment to the adluminal compartment without disruption of the BTB [2]. Furthermore, Dym and Cavicchia reported development of germ cells in the intermediate compartment, separation of the BTB on the luminal side and safe passage of the germ cells through to the adluminal compartment to complete meiosis [3]. The BTB situated above the spermatocyte (Fig. 6C) is also opened physically by meiosis division of the spermatocyte (Fig. 6D; see Figs. 1C and 2A). Although the pre-existing BTB above the spermatocyte underwent dissolution according to Russell’s theory [2], it is reasonable to speculate that the ES associated with early spermatids is the counterpart of the open BTB. Furthermore, the ES associated with developing spermatids was also recognized (Figs. 6E and 6F; see Figs. 1D, 1E, 2B, and 2C). Dissociation of inter-Sertoli cell junctions into hemijunctions has also been suggested [3, 40].

Figure 7 shows a schematic drawing illustrating the circulation of ES between adjacent Sertoli cells, Sertoli-germ cells and different stages of germ cell development in the seminiferous epithelium. After cell division of the spermatagonia, newborn spermatocytes may be elevated with the BTB (Fig. 5A). The ES lying against the BM of the seminiferous epithelium (Fig. 3A) may be transported (Fig. 3B), may assemble the BTB below the spermatocyte (Fig. 3C) and may organize the intermediate compartment (Fig. 1B). The BTB may be opened physically by meiosis division of the spermatocyte, and then the ES that is the counterpart of the BTB may attach to early spermatids (Fig. 1C) late spermatids (Fig. 1D) and mature spermatids (Fig. 1E). After spermatia, ES may be located on the apical surface of the seminiferous epithelium (Fig. 1F). It has been suggested that the possible formation of pools of free hemijunctions, namely ES, which recycle into earlier germ cells, may be involved in formation of basal inter-Sertoli cell junctions [40], and this paper supports the hypothesis that the ES unit circulates in the seminiferous epithelium.

Many studies since the 1960s have focused on the morphological events that take place in the seminiferous epithelium. Recent advances in biochemistry and molecular biology have shifted attention to understanding some of the key events that regulate spermatogenesis, such as opening and closing of the BTB to permit timely passage of primary spermatocytes across the BTB [4–8, 35–37]. Though the results of recent findings in this area also suggest that the BTB in the seminiferous epithelium must “open” to accommodate migration of primary spermatocytes from the basal compartment to the adluminal compartment, the BTB probably maintains its integrity to protect haploid germ cells against the autoimmune system. Overall, analyses using biochemical methods or techniques of molecular biology alone cannot fully unravel the physiological meaning of the BTB, nor have they been able to uncover the presence of an intermediate compartment.

The seminiferous epithelium consists of two distinct populations: Sertoli cells and spermatogonia and their proliferating progeny that move upwards in the epithelium as they differentiate into spermatzoa. The Sertoli cells provide mechanical support for germ cells, and by changes in their shape, they no doubt contribute to upward movement of the differentiating germ cells and release of the spermatzoa into the lumen. Further research is necessary to prove this model.

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