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Review

Emergent roles for intercellular adhesion molecule-1 in the restructuring of the blood-testis barrier during spermatogenesis in the mammal

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Summary. Mammalian spermatogenesis is comprised of a series of molecular, cellular, and morphological events that underscore the movement of developing germ cells across the blood-testis barrier. These events involve the restructuring of tight junctions, basal ectoplasmic specializations, gap junctions, and desmosomes, which constitute blood-testis barrier function. Previous studies show that preleptotene/leptotene spermatocytes traverse the blood-testis barrier while transiently trapped within an intermediate compartment, which sequesters primary spermatocytes away from basal and adluminal compartments of the seminiferous epithelium. Preleptotene/leptotene spermatocytes enter the adluminal compartment when stable junctions ahead of spermatocytes disassemble, while new junctions assemble behind them. While there is enormous restructuring of the seminiferous epithelium, the mechanism of germ cell movement is incompletely understood. In this perspective, the significance of intercellular adhesion molecule-1 in the restructuring of the blood-testis barrier during spermatogenesis in the mammal is discussed.

Key words: Testis, Spermatogenesis, Cell junction, Blood-testis barrier, Germ cell movement

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Introduction

Spermatogenesis, the process by which sperm are produced, takes place within the seminiferous tubule, the fundamental unit of the mammalian testis, and the entire process spans 51.6 days in the rat [for reviews, (de Kretser and Kerr, 1988; Kerr et al., 2006; Schlatt and Ehmcke, 2014)]. Sperm production begins with the renewal or differentiation of spermatogonial stem cells, which either restores the stem cell pool or yields spermatogonia connected by cytoplasmic bridges, respectively [for reviews, (de Rooij and Russell, 2000; Oatley and Brinster, 2008; Greenbaum et al., 2011)]. Thereafter, differentiating spermatogonia undergo several synchronized mitotic divisions before giving rise to primary spermatocytes. This event is followed by meiosis I, which produces secondary spermatocytes, and then by meiosis II, which produces spermatids. In the final phase of spermatogenesis, spermatids undergo several changes that essentially transform round spermatids into elongated spermatids. Spermatozoa ultimately detach from the seminiferous epithelium and enter the epididymis for further maturation [for reviews, (Russell, 1993a; Kerr et al., 2006; Robaire et al., 2006; O'Donnell et al., 2011)].

Spermatogenesis is divided into 14 distinct stages (i.e., I to XIV) in the rat. Together, these stages constitute the seminiferous epithelial cycle [(LeBlond and Clermont, 1952; Hess, 1990); for reviews, (Russell et al., 1990; Hess and Renato de Franca, 2008)] (Fig. 1). In a typical cross-section of the adult testis stained with either periodic acid-Schiff or haematoxylin and eosin, Sertoli cells are observed with their cytoplasmic stalks

extending from the base of the seminiferous tubule to its apex. Sertoli cells connect to each other by basally-located cell junctions, which create the blood-testis barrier (also known as the Sertoli cell barrier) [(Dym and Cavicchia, 1977; Russell, 1978); for reviews, (Setchell, 1980; Russell, 1993b)]. The blood-testis barrier divides the seminiferous epithelium into the basal compartment, in which spermatogonia and primary spermatocytes (i.e., preleptotene spermatocytes) reside; and the adluminal compartment, in which the remaining germ cells reside (Fig. 2). Thus, each stage contains 4-5 generations of developing germ cells that are supported by nurse-like Sertoli cells. The main distinction between the stages, however, is in the types of germ cells that occupy each stage (Fig. 1). In this way, the 14 stages of the seminiferous epithelial cycle can be easily discerned.

As germ cells develop, they move from the base of the seminiferous tubule to its apex, indicating primary spermatocytes traverse the blood-testis barrier during the seminiferous epithelial cycle. Morphological studies demonstrate that primary spermatocytes (i.e., preleptotene/leptotene spermatocytes) cross the barrier at stages VIII to XI while enclosed within the intermediate compartment, which sequesters spermatocytes away from basal and adluminal compartments of the seminiferous epithelium (Dym and Cavicchia, 1977; Russell, 1978); for a review, (Russell, 1993b) (Fig. 2). The intermediate compartment is created by Sertoli cell junctions that restructure during germ cell movement, which initiates when stable junctions in front of moving germ cells disassemble and nascent ones assemble at the back of cells. Thus, the blood-testis barrier is never completely disassembled and never completely assembled during germ cell movement. While there is enormous restructuring of the seminiferous epithelium, the mechanism of germ cell movement is incompletely understood. In this perspective, the significance of intercellular adhesion molecule-1 (ICAM-1) in the restructuring of the blood-testis barrier is presented.

Structure and function of the blood-testis barrier

The blood-testis barrier, a physical barrier that is created by Sertoli cell junctions, divides the seminiferous epithelium into a basal compartment and an adluminal compartment [for reviews, (Setchell, 1980; Waites and Gladwell, 1982; Kerr et al., 2006; Cheng and Mruk, 2012). It is one of the tightest blood-tissue barriers in the mammalian body based on early studies that showed the testis to be impenetrable to intravenously injected dyes (Ribbert, 1904; Bouffard, 1906). The blood-testis barrier has three important

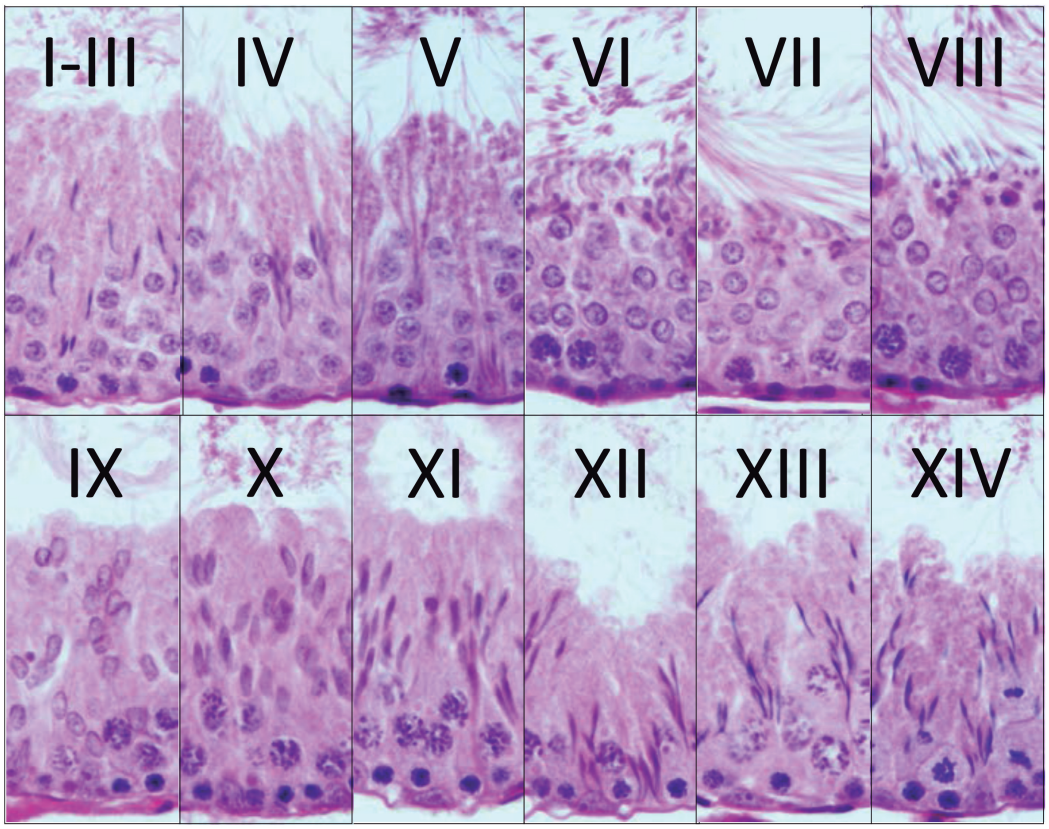

Fig. 1. Fourteen stages of the seminiferous epithelial cycle in the rat. A Bouin's-fixed adult testis was embedded in paraffin wax and sectioned, and the cross-sections were stained with haematoxylin and eosin. Each image shows a portion of a seminiferous tubule at one of fourteen stages. Stages I-III are difficult to discern. Thus, they were grouped into a single image.

functions. It prevents the entry of unwanted substances into the adluminal compartment, regulates the passage of needed substances into the same compartment, and sequesters meiotic germ cells away from the immune system. Spermatogonia and preleptotene spermatocytes, however, are exposed to the immune system.

Blood-testis barrier function is largely constituted by tight junctions and ectoplasmic specializations, with desmosomes and gap junctions concentrating between areas occupied by the former cell junction types [for reviews, (Cheng and Mruk, 2002; Vogl et al., 2008)]. While tight junctions, desmosomes, and gap junctions are typical of most epithelial cells, ectoplasmic specializations are specific to the testis. Ectoplasmic specializations are hybrid-like anchoring junctions that are characterized by the presence of actin microfilament bundles situated between the Sertoli cell plasma membrane and the cisterna of the endoplasmic reticulum [for reviews, (Mruk and Cheng, 2004; Vogl et al., 2008)]. There are two types of ectoplasmic specializations. Ectoplasmic specializations present between Sertoli cells at the blood-testis barrier are defined as basal ectoplasmic specializations, whereas those between Sertoli cells and step 8/post-step 8 spermatids are defined as apical ectoplasmic specializations. While the ectoplasmic specialization is comprised of several structural and signaling proteins, many of these proteins are found within other cell junction types. For example, focal adhesion kinase (FAK), a non-receptor protein tyrosine kinase that predominantly localizes to focal adhesions, sites of cell-extracellular matrix contact, regulates the actin cytoskeleton, cell adhesion, and cell movement in normal and tumorigenic cells [for reviews, (Hanks and Polte, 1997; Parsons, 2003)]. In the testis, however, FAK associates with β1-integrin at the apical ectoplasmic specialization and occludin at tight junctions (Mulholland et al., 2001; Siu et al., 2009a,b; Lie et al., 2012). Taken collectively, these findings illustrate that the blood-testis barrier is one of the most unique blood-tissue barriers in the mammalian body.

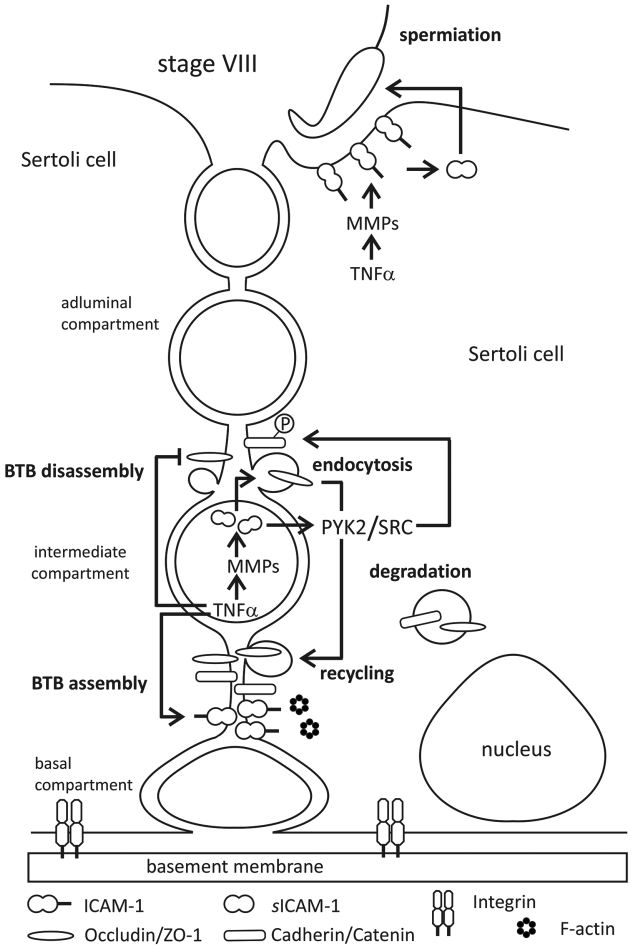

Fig. 2. Hypothetical model of ICAM-1 and sICAM-1 function in the testis. The blood-testis barrier divides the seminiferous epithelium into a basal and an adluminal compartment. A preleptotene/leptotene spermatocyte within the intermediate compartment is shown. Preleptotene/leptotene spermatocytes enter the adluminal compartment when stable junctions ahead of spermatocytes disassemble, while nascent junctions assemble behind them via a mechanism that involves protein endocytosis, recycling and/or degradation. Thus, the blood-testis barrier is never completely disassembled and never completely assembled during germ cell movement. These events are partly mediated by pro-inflammatory cytokines such as TNFα, which regulates ICAM-1 to bring about the assembly or disassembly of the blood-testis barrier. For example, MMPs can cleave ICAM-1 to generate sICAM-1, which mediates blood-testis barrier disassembly and possibly spermiation. Non-receptor protein tyrosine kinases such as PYK2 and SRC also participate in these events.

Intercellular adhesion molecules

Introduction

There are interesting similarities in the way different cells cross epithelial or endothelial barriers under normal and pathological conditions. During inflammation/infection, leukocytes cross the endothelial barrier while enclosed within the lateral border recycling compartment, a dynamic chamber of interconnected vesicle-like structures proximal to and contiguous with the plasma membrane [(Mamdouh et al., 2003, 2008, 2009); for a review (Muller, 2011)]. This channel is lined with immunoglobulin (Ig)-like cell adhesion proteins such as ICAM-1, junctional adhesion molecule-A (JAM-A), and coxsackie and adenovirus receptor that seal the endothelial barrier as leukocytes cross it. Because this compartment is somewhat similar to the intermediate compartment in the testis, it can provide important insights on how germ cells cross the blood-testis barrier or, at least, provide clues on how it can be studied. For example, coxsackie and adenovirus receptor encircles preleptotene spermatocytes as they traverse the blood-testis barrier (Mirza et al., 2007), revealing Ig-like adhesion proteins seal germ cells within the intermediate

compartment (Fig. 2). Another similarity between systems is that Ig-like adhesion proteins are internalized and then possibly recycled back to the plasma membrane during cell movement [for reviews, (Reglero-Real et al., 2012; Vestweber et al., 2014; Xiao et al., 2014)]. Presently, it is not clear if other mechanisms are also involved.

Structure and function of intercellular adhesion molecule-1

ICAMs are Ig-like cell adhesion and signaling proteins that are expressed by different cells that include leukocytes, macrophages, dendritic cells, endothelial cells, and epithelial cells. Five members (ICAMs-1 to -5) have been characterized and found to contain an N-terminal extracellular domain, a transmembrane domain, and a C-terminal cytoplasmic domain [(Tian et al., 1997; Hermand et al., 2003); for reviews (Toivanen et al., 2008; Lawson and Wolf, 2009)]. ICAM-1, the best-studied member, facilitates cell adhesion by binding β2-integrins (Burns et al., 2001; Sadowska et al., 2004; Porter and Hall, 2009). ICAM-1 also binds other proteins that include JAM-A, cadherin, actin, β-tubulin, ezrin, moesin, α-actinin, filamin B, cortactin, proto-oncogene tyrosine-protein kinase Src (c-SRC), and proline-rich tyrosine kinase 2 (PYK2) [(Carpen et al., 1992; Federici et al., 1996; Casasnovas et al., 1998; Heiska et al., 1998; Barreiro et al., 2002; Ostermann et al., 2002; Tilghman and Hoover, 2002; Carman et al., 2003; Celli et al., 2006; Molock and Lillehoj, 2006; Kanters et al., 2008); for reviews, (Rahman and Fazal, 2009; Muller, 2011)]. While *Icam1* null mice are viable and fertile, there are widespread defects during inflammation (e.g., a decrease in inflammatory cell infiltration, an increase in neutrophil count, a delay in wound healing) compared to wild-type littermates (Sligh et al., 1993; Kitagawa et al., 1998; Nagaoka et al., 2000; Hallahan et al., 2002; Dunne et al., 2003). Most ICAM studies have been performed on cells from the immune system interacting with endothelial cells.

Regulation of intercellular adhesion molecule-1

ICAM-1 clustering, which occurs during the early stages of leukocyte movement across the endothelium, involves the recruitment of signaling proteins and the activation of signaling cascades [(Barreiro et al., 2002; Carman and Springer, 2004); for a review (Muller, 2011)]. ICAM-1 clustering also results in the recruitment of cytoskeletal proteins, the translocation of ICAM-1 to sites of cell-cell contact, and the formation of stress fibers. For example, ICAM-1 clustering leads to the activation of Rho GTPases and the phosphorylation of focal adhesion proteins such as FAK, cortactin, paxillin, and CRK-associated substrate [(Thompson et al., 2002; Greenwood et al., 2003; Yang et al., 2006; Vestweber et al., 2013); for reviews, (Wittchen, 2009; Heemskerk et al., 2014)], indicating GTPases act downstream of ICAM-1. ICAM-1 clustering can also be induced by function-blocking monoclonal antibodies (Tilghman and Hoover, 2002; Millan et al., 2006; van Buul et al., 2010; Sumagin et al., 2011), illustrating this phenomenon can be recreated and studied *in vitro*. While the ICAM-1 level is generally low in most cells, it is upregulated by pro-inflammatory cytokines such as tumor necrosis factor α (TNFα), interleukin-1, and interferon-γ (Dustin et al., 1986; Becker et al., 1991; Fonsatti et al., 1997; Spoelstra et al., 1999; Thomson et al., 1999; Hosokawa et al., 2006; Defrere et al., 2008; Inoue et al., 2011). In Sertoli cells, TNFα increases the ICAM-1 level via the c-Jun N-terminal kinase/stress-activated protein kinase signaling pathway [(Ricciolo et al., 1995; Ziparo et al., 1995; De Cesaris et al., 1998; Lydka et al., 2012); for a review, (Nishina et al., 2004)]. Other studies show that cytokines can activate matrix metalloproteases (MMPs), which cleave the extracellular domain of ICAM-1 to generate soluble ICAM-1 (sICAM-1) [(Lyons and Benveniste, 1998; Fiore et al., 2002; Sultan et al., 2004; Sithu et al., 2007; Essick et al., 2008; Pino et al., 2009); for a review (Lawson and Wolf, 2009)]. sICAM-1 has signaling properties [for a review, (Lawson and Wolf, 2009)].

Intercellular adhesion molecule-1 in the testis

Icam-1 is expressed by cells of the testis, where it associates with Sertoli and germ cells at all stages of the seminiferous epithelial cycle (Xiao et al., 2012). Interestingly, ICAM-1 immunoreactivity is highest at the blood-testis barrier at stage VIII, coinciding with germ cell movement across the barrier. Furthermore, ICAM-1 partially colocalizes and co-immunoprecipitates with occludin and zona occludens-1 (ZO-1, both are tight junction proteins), as well as with N-cadherin and β-catenin (basal ectoplasmic specialization proteins). Taken collectively, these findings illustrate that ICAM-1 is a component protein of the blood-testis barrier (Fig. 2). Equally important, ICAM-1 overexpression in Sertoli cells improves barrier function as determined by the transepithelial electrical resistance method (Xiao et al., 2012). Thus, ICAM-1 is involved in the establishment of the blood-testis barrier and/or the maintenance of its integrity during spermatogenesis. While ICAM-1 is highest at the blood-testis barrier, it is also weakly present throughout the seminiferous epithelium (Xiao et al., 2012), illustrating its function goes beyond that at the blood-testis barrier.

In addition to ICAM-1, normal and tumorigenic cells can also generate sICAM-1, which lacks transmembrane and cytoplasmic domains, via cleavage of ICAM-1 [for a review, (Lawson and Wolf, 2009)]. In the testis, the level of sICAM-1 is highest in germ cells, indicating they have the ability to cleave ICAM-1. Furthermore, sICAM-1 overexpression disrupts Sertoli cell barrier function (Xiao et al., 2012) (Fig. 2). The levels of N-cadherin and γ-catenin, but not those of α- and β-

catenin, decrease after overexpression, suggesting cell-cell adhesion is disrupted. γ-Catenin, a member of the armadillo protein family, localizes to both the desmosome and adherens junction in epithelial cells, and it can replace β-catenin within the adherens junction [for reviews (Zhurinsky et al., 2000; Kowalczyk and Green, 2013)]. Presently, it is not known which pool of γ-catenin is affected by sICAM-1 overexpression. The level of connexin 43, a component protein of the gap junction, as well as the levels of c-SRC/c-Src^{Y530} and PYK2/PYK2^{Y402}, also decrease after overexpression. Interestingly, connexin 43 binds plakophilin-2, a component protein of the desmosome [for reviews, (Hatzfeld, 2007; Bass-Zubek et al., 2009)]. This interaction is critical for barrier function because knockdown of both connexin 43 and plakophilin-2 affects the integrity of the Sertoli cell barrier (Li et al., 2009).

These *in vitro* findings are corroborated by sICAM-1 overexpression studies *in vivo*. For example, sICAM-1 overexpression in the testis results in the loss of spermatocytes and round spermatids from the seminiferous epithelium, as well as in the misorientation of elongated spermatids (Xiao et al., 2012). Spermatocytes and round spermatids attach to Sertoli cells via desmosomes, indicating there is crosstalk between multi-protein complexes and signaling cascades across different cell junctions. Furthermore, the integrity of the blood-testis barrier is compromised in these seminiferous tubules as assessed by the diffusion of vascularly-infused inulin-fluorescein isothiocyanate into the adluminal compartment of the seminiferous epithelium (Xiao et al., 2012). Taken collectively, these findings illustrate that sICAM-1 is involved in the disassembly of stable junctions ahead of preleptotene/leptotene spermatocytes, whereas ICAM-1 promotes the assembly of new junctions behind them (Fig. 2).

Future perspectives and concluding remarks

In this review, the significance of ICAM-1 in the restructuring of the blood-testis barrier was discussed. ICAM-1 and sICAM-1 have opposite roles at the blood-testis barrier. While ICAM-1 promotes the assembly of new junctions behind preleptotene/leptotene spermatocytes, sICAM-1 facilitates the disassembly of stable junctions ahead of spermatocytes. Presently, it is not clear if other members of this protein family also facilitate germ cell movement across the blood-testis barrier. For example, the loss of ICAM-2 disrupts leukocyte transmigration *in vitro* and *in vivo* (Gerwin et al., 1999; Huang et al., 2006; Porter and Hall, 2009). In the testis, however, ICAM-2 does not localize to the blood-testis barrier. Instead, it is present at the apical ectoplasmic specialization (Xiao et al., 2013). It is also important to determine how sICAM-1 activates intracellular signaling cascades. Together, these studies provide new information on the regulation of the blood-testis barrier during spermatogenesis.

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