Chapter 7
What are the multiple roles of the Sertoli cell?
Supporting spermatogenesis and immune regulation

Jannette M. Dufour

Introduction
The primary function of the testis is to produce testosterone and sperm. The testis is divided into two compartments: the interstitium and the seminiferous tubules (Fig. 1). The interstitium is located between the seminiferous tubules and contains Leydig cells, immune cells, blood vessels, and lymphatic vessels. Leydig cells are responsible for producing the male sex hormone testosterone, which is secreted from the testis and is critical for the development of male external genitalia and initiation of spermatogenesis. Sperm production occurs in the seminiferous tubules. The seminiferous tubules contain Sertoli cells and germ cells and are surrounded by peritubular myoid cells (Fig. 1). Sertoli cells are sustentacular cells that were first described by Enrico Sertoli in 1865. They extend from the base to the lumen of the seminiferous epithelium and have been described as nurse cells as they engulf the germ cells and provide an appropriate microenvironment for germ cell development.

At puberty, testosterone levels rise, triggering the onset of spermatogenesis. At this time, the spermatogonia progress to primary spermatocytes and the advanced germ cells (spermatocytes and spermatids) first appear (Fig. 1). During spermatogenesis, germ cells undergo three stages of development: mitosis (spermatogonial proliferation), meiosis (spermatocyte DNA recombination, reduction and division) and spermiogenesis (spermatid differentiation). After differentiation the sperm are released into the lumen and residual bodies (containing excess spermatid cytoplasm) are phagocytosed by the Sertoli cells. Since the advanced germ cells are not present at birth when immune self-antigen tolerance is established, there is potential for an immune response to be generated against the germ cells that could result in autoimmune orchitis (testis inflammation and anti-sperm antibodies), loss of germ cells and male infertility.

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Role in spermatogenesis

In addition to providing structural support and creating the appropriate environment for germ cell development, Sertoli cells provide a plethora of factors important for spermatogenesis including growth factors, hormones, metabolites, nutrients, adhesion molecules, proteases, retinoic acid, and iron to name a few. The precise time when these factors are produced is important for maintaining the cycle of the seminiferous epithelium.

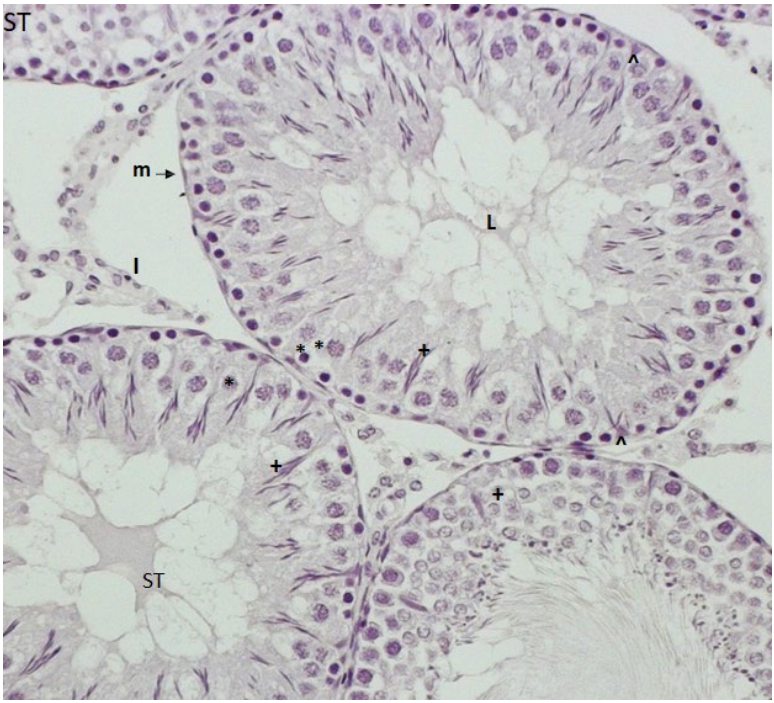

Figure 1. Organization of the testis. The testis is divided into the seminiferous tubules (ST) and the interstitial space (I). The seminiferous tubules are surrounded by peritubular myoid cells (m) and contain the germ cells and Sertoli cells (caret symbol points to Sertoli cell nuclei). *, spermatocyte; +, round and elongated spermatids; L, lumen.

The various different germ cells present in the testis are organized into the cycle of the seminiferous epithelium, which is defined by the specific germ cells present in a given cross section of the tubule. The timing of this cycle varies between species and is controlled by the

germ cells. This was shown by transplantation of rat germ cells into mouse testes, which led to incorporation of the timing of the rat cycle in mice thus demonstrating that the germ cells are responsible for the timing of the cycle of the seminiferous epithelium. Consistently, at least in mice, Sertoli cells are present in two different transcriptional states that are dependent on the presence of germ cells. These states correlate with high and low retinoic acid levels and suggest that germ cells control the timing of the expression of specific factors by Sertoli cells that are necessary at specific times of their development.

Besides their roles in testis formation during embryonic development, cell ablation studies confirmed the role of Sertoli cells in testis and germ cell development postnatally. Loss of Sertoli cells led to loss of all germ cells and spermatogenesis. Additionally, Sertoli cells are necessary to maintain the differentiated state of peritubular myoid cells before but not after puberty and for peritubular myoid cell function in the adult. Sertoli cells are also necessary for adult Leydig cell development and survival. These studies further support the critical role of Sertoli cells in spermatogenesis and expand their importance to regulation of the other testicular cells.

Physical, physiological and immunological components of the blood-testis-barrier

The blood-testis-barrier (BTB) is also formed at puberty. However, unlike other blood tissue barriers, the BTB is not located at the blood vessels but instead is located within the seminiferous tubules toward the basal side of the Sertoli cells. It consists of the body of the Sertoli cells and the tight junctions formed between adjacent Sertoli cells and separates the seminiferous epithelium into the basal and adluminal compartments (Fig. 2).

The BTB has physical, physiological and immunological functions that work together to prevent germ cell exposure to toxins, create the appropriate milieu for germ cell development and prevent an autoimmune response to the advanced germ cells. The physical and physiological components act together to control the movement of molecules across the barrier. The physical barrier restricts the passage of some molecules, while the physiological part contains specific transporters located along the basal and apical membranes of the Sertoli cells and regulates the movement of factors in or out of the lumen. In this way the Sertoli cells are able to

create the appropriate environment for germ cell development. Additionally, the immunological part functions to prevent entry of antibodies and, together with the peritubular myoid cells, the BTB prevents entry of immune cells into the seminiferous tubules.
One interesting aspect of the BTB is the need to both protect the germ cells from an immune response (and maintain the microenvironment for germ cell development), and yet at the same time allow the germ cells to cross from the basal to adluminal

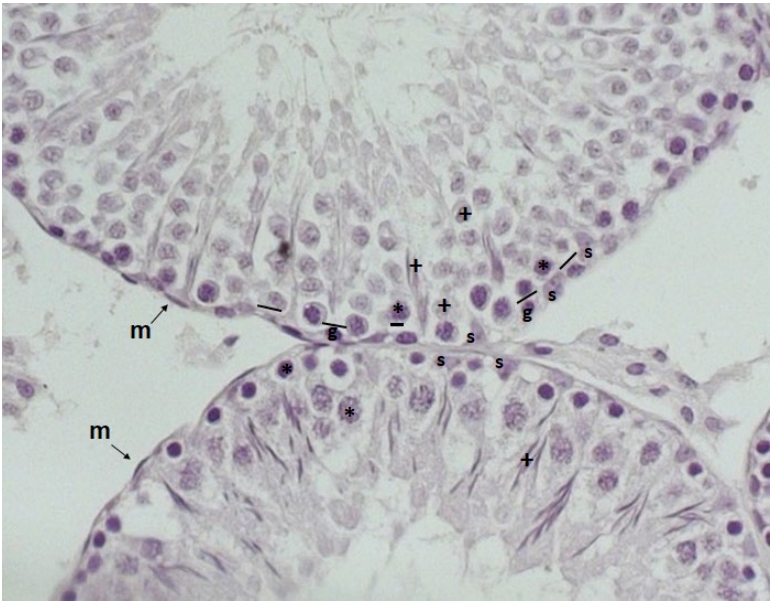

Figure 2. Seminiferous epithelium. The BTB (lines represents TJ) between adjacent Sertoli cells separates the seminiferous epithelium into the basal (containing spermatogonia and preleptotene spermatocytes) and adluminal (containing spermatocytes and spermatids) compartments. m, peritubular myoid cells; s, Sertoli cell; g, spermatogonia; *, spermatocyte; +, round and elongated spermatids.

compartment without exposing the contents of the adluminal compartment to the immune system. This process requires unique coordination. As the preleptotene spermatocytes progress to leptotene spermatocytes, they migrate away from the basal edge of the tubules. At this time, new tight junctions form below them, creating an intermediate compartment with the old tight junctions above and new tight junctions below. This process is partially

coordinated by testosterone and cytokines such as TNFα and TGFβ 2 and 3. Testosterone promotes the formation of new tight junctions below the spermatocytes, while the cytokines disrupt the BTB causing the disassembly of the old tight junctions. This transient intermediate compartment allows the spermatocytes to cross the BTB without opening the adluminal compartment to the interstitial space. Consequently, the BTB is a dynamic barrier, constantly breaking down at some points and being remade at others.

Sertoli cell as immune regulators

Given the immunogenic nature of the germ cells, the testis must be able to prevent an immune response to protect the germ cells. At the same time, bacteria and viruses can infect the testis, and therefore the testis must also be able to mount an antimicrobial response to prevent infections. However, if the inflammation associated with the antimicrobial response is not held in check, it can lead to an autoimmune response and male infertility. Conversely, if the testis fails to mount a robust antimicrobial response, the testis can become a sanctuary site for pathogens. While numerous components allow the testis to both provide germ cell protection and inhibit infections, here we will focus on the role of the Sertoli cells.

The initial evidence for Sertoli cell involvement in testis immune regulation comes from transplantation studies. Transplantation of tissue from genetically different individuals (allogeneic) or from a different species (xenogeneic) usually results in immune rejection of the grafted tissue within a few weeks. On the contrary, when allogeneic or xenogeneic tissue is transplanted into the testis, it enjoys prolonged survival. Elimination of Leydig cells and germ cells demonstrates the importance of Sertoli cells in this immune protection as foreign tissue grafts still survived long-term in the absence of Leydig cells or germ cells. Moreover, transplantation of isolated Sertoli cells as allografts or xenografts to an ectopic site outside the testis, had long-term survival without the need for immune suppressing drugs. Also, co-transplantation of Sertoli cells with allogeneic or xenogeneic tissue, such as pancreatic islets, prolongs the survival of the co-grafted cells. Lastly, ablation of Sertoli cells from the testis showed that Sertoli cells are required to form the BTB, which is necessary to prevent the entry of antibodies and immune cells into the adluminal compartment of the seminiferous epithelium, although the remaining peritubular myoid cells were capable of blocking the entry of macrophages into the tubules.

As mentioned above, one mechanism to prevent an immune response is to sequester the germ cells behind the BTB. However, there is more to immune protection of the germ cells. Recently, it was found that not all germ cell antigens are sequestered and instead some antigens are localized to residual bodies where they are phagocytosed by Sertoli cells, exported from the seminiferous tubules and present in immune complexes in the interstitial space. This leads to induction of regulatory T cell (Tregs) immune tolerance. Tregs were found to be critical for this tolerance as Treg depletion resulted in autoimmune orchitis. In addition, Sertoli cells express several immunomodulatory factors that can alter the immune cell response towards tolerogenic. For instance, they express immunoregulatory factors (TGFβ, IDO, activin A, IL6, galectin 1, IGF1), complement inhibitory proteins (C1INH, CD46, CD55, CD59 and clusterin), and anti-apoptotic proteins (serpina3n, serpinb9). These factors can influence macrophages (M2), T cells (Tregs) and dendritic cells (tolerogenic DC) to be immune protective. For example, Sertoli cell expression of galectin 1 has been implicated in the production of tolerogenic DC that in turn induce Tregs and Sertoli cell production of TGFβ and IDO has also been connected to Treg induction.

Under other circumstances, Sertoli cells can shift to an antimicrobial response and express inflammatory mediators to fight off infections. Sertoli cells express inflammatory cytokines (IL1β, IL6, TNFα, MCP1), pattern recognition receptors (TLR 2, 3, 4, 5, and 6, NOD 1 and 2), NFκB and antimicrobials (β-defensin, α-defensin, PKR). In response to viruses and bacteria, pattern recognition receptors initiate signaling cascades like NFκB. NFκB activation leads to increased expression of the inflammatory cytokines and antimicrobials mentioned above, which promotes immune cell migration and activation (M1 macrophages, dendritic cells, Th1 and Th17 CD4 T cells, and CD8 CTL T cells). Sertoli cells can also express TAMs (Tyro3, Axl and Mer). TAMs suppress TLR signaling pathways and decrease inflammation. Together this allows the Sertoli cells to provide the delicate balance necessary to protect the germ cells and fight off pathogens.

Bacteria and at least twelve viruses have been detected in the testis. Several bacteria were found to infect the male reproductive tract but it is very rare for bacteria to reach the testes. In contrast, viral infections are much more common and it has now been confirmed that the testis can act as a viral sanctuary site for viruses that can be sexually transmitted. Not only is this a concern from an

infection stand point but viral infection induces an inflammatory response that leads to germ cells loss. Marburg, Zika, mumps, HIV, and SARS-CoV-2 can infect testicular cells, and all except HIV, and SARS-CoV-2 have been shown to infect Sertoli cells. Persistent viral infection activates TLRs and initiates an inflammatory response that alters BTB TJ proteins and increases BTB permeability, ultimately leading to loss of germ cells. Although rare, the testis can also serve as a reservoir site for relapse of acute lymphoblastic leukemia. Together this emphasizes the need to increase our understanding of testicular, including Sertoli cell, immune regulation.

Conclusions

Sertoli cells play important roles in supporting spermatogenesis and controlling testicular immune regulation. They provide the structural support for the three stages of germ cell development and create the BTB with physical, physiological and immunological functions. This allows them to maintain the appropriate milieu necessary for germ cells to progress through meiosis and spermiogenesis. At the same time Sertoli cells regulate the immune response to both protect germ cells from the immune system and also provide an antimicrobial response to prevent infection. If this is not properly controlled, it can result in an autoimmune response against the germ cells or provide a sanctuary site for pathogens.

Suggested reading

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