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Sertoli cells- Immunological sentinels of spermatogenesis

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Abstract

Testicular germ cells, which appear after the establishment of central tolerance, express novel cell surface and intracellular proteins that can be recognized as ‘foreign antigens’ by the host’s immune system. However, normally these germ cells do not evoke an auto-reactive immune response. The focus of this manuscript is to review the evidence that the Blood-Testis-Barrier (BTB)/Sertoli cell (SC) barrier along with the SCs ability to modulate the immune response is vital for protecting auto-antigenic germ cells. In normal testis, the BTB/SC barrier protects the majority of the auto-antigenic germ cells by limiting access by the immune system and sequestering these ‘new antigens’. SCs also modulate testis immune cells (induce regulatory immune cells) by expressing several immunoregulatory factors, thereby creating a local tolerogenic environment optimal for survival of nonsequestered auto-antigenic germ cells. Collectively, the fortress created by the BTB/SC barrier along with modulation of the immune response is pivotal for completion of spermatogenesis and species survival.

Keywords

Testis; Blood-Testis-Barrier; Immune privilege; Sertoli cells; Spermatogenesis

The mammalian testis consists of seminiferous tubules surrounded by interstitial tissue, which includes Leydig cells, blood vessels, leukocytes and fibroblasts. Spermatogenesis takes place in the seminiferous tubules which are composed of Sertoli cells (SCs) and maturing germ cells surrounded by one (rodents) or more (large animals) layer(s) of peritubular myoid cells (Fig. 1a) [1, 2]. The majority of the germ cells need immune protection as they first appear after systemic tolerance is established and express novel cell surface and intracellular antigens that can elicit an immunological response. SCs protect these auto-antigenic germ cells by forming the Blood-Testis-Barrier (BTB)/SC barrier and expressing immunoregulatory factors, thereby creating an immune privileged environment which is a prerequisite for the survival of testicular germ cells (Fig. 1a). In this review, we

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will focus on the immunological aspects of SCs and the BTB/SC barrier in protecting spermatozoa.

1. Spermatogenesis

Spermatogenesis is a complex and highly organized process where germ cells undergo three phases of development: mitosis (spermatogonial proliferation), meiosis (spermatocyte DNA recombination, reduction and division), and spermiogenesis (spermatid differentiation), resulting in the transformation of the undifferentiated spermatogonia into highly specialized spermatozoa [3]. In rodents, the spermatogonial population is divided into two main categories- 1) undifferentiated consisting of type A_{single} , A_{paired} and A_{aligned} and 2) differentiated consisting of A1-A4, Intermediate and type B [3, 4]. In primates and humans, the spermatogonial hierarchy consists of two main populations of type A undifferentiated spermatogonia- A_{dark} and A_{pale} . The A_{pale} spermatogonia undergo mitotic divisions to produce type B1 spermatogonia [3, 4]. In all mammals, the type B/B1 spermatogonia undergo mitosis to give rise to the cells that represent the beginning of meiosis, the preleptotene spermatocytes. The preleptotene spermatocytes enter prophase I of meiosis, transform into leptotene, zygotene, pachytene and diplotene cells, which then quickly finish meiosis I forming secondary spermatocytes. While advancing through the long meiotic prophase I, spermatocytes undergo several changes such as chromosome condensation, genetic recombination and migration from the basal to the adluminal compartment of the seminiferous tubule through the BTB/SC barrier [3]. The second meiotic division, meiosis II, rapidly follows meiosis I to produce haploid cells, the spermatids [3, 4]. Spermatids differentiate into spermatozoa by proceeding through spermiogenesis, before being released into the lumen [3]. These meiotic and post-meiotic germ cells, which first appear around puberty, express novel antigens that can be recognized as ‘foreign antigens’ by the mature immune system [5, 6]. Completion of spermatogenesis is pivotal for species survival; therefore the testicular environment is specialized to prevent an autoimmune response that could be generated by these ‘foreign antigens’.

2. Immune Privilege and Sertoli Cells

Immune privileged sites are places in the body where foreign antigens are tolerated without evoking detrimental inflammatory immune responses. The immune privilege status of the testis is well recognized since the auto-immunogenic germ cells reside within the testis without generating an immune response. Exploration of anatomical, physiological and cellular components of the testis indicated that of all the testicular components, SCs are of keen importance in testis immune privilege [7, 8].

SCs were first described by Enrique Sertoli in 1865. He referred to the SC as a ‘sustentacular cell’ as they provide structural and functional support for the development of the maturing germ cells [9]. In addition, SCs protect the auto-immunogenic germ cells from the host’s immune system by the BTB/SC barrier that includes tight junctions between adjacent SCs, together with the body of the SC (Fig. 1a) [10]. Besides serving as a wall between maturing germ cells and immune cells, SCs also have the capacity to modulate the immune response by expressing several immunoregulatory factors. Here, we will discuss

both components of the SCs, the BTB/SC barrier and immune modulation as they relate to testis immune privilege and spermatogenesis.

3. Blood-Testis-Barrier

Blood-tissue-barriers restrict or control the entry of molecules or cells present in circulation from entering a tissue or compartment. Common examples are the Blood-Brain-Barrier (BBB), Blood-Placenta-Barrier, Blood-Thymus-Barrier and Blood-Testis-Barrier. Evidence for the existence of a “barrier” in the testis was first pioneered by experiments in the early 1900s demonstrating the exclusion of injected dyes from the testicular tissue. Despite the early recognition of this “barrier”, its presence was largely ignored until 1950s when a series of papers further confirmed these original observations by showing that certain dyes or radiolabelled material did not pass readily into the seminiferous tubules [11, 12]. Chiquoine (1964) appears to be the first person to name this testis barrier as the “Blood-Testis-Barrier”. He further suggested that this BTB is open at times while closed at others based on De Bruyn’s observation of sporadic staining of fluorescent dyes in the testis [11, 13]. Due to the functional similarity between the BBB and BTB, it was speculated that like the BBB the BTB is formed by tight junctions between endothelial cells lining the blood vessels, which restrict the exist of molecules from the blood. Initial observations of weakly stained interstitial nuclei and increased fluorescence in interstitium after disruption of blood vessels by CdCl_2 treatment suggested the presence of the BTB at the testicular capillaries [14, 15]. The testis endothelial expression of several markers associated with barrier properties of brain vessels such as the glucose transporter, P-glycoprotein, γ -glutamyl transpeptidase and endothelial barrier antigen further supported this notion [16, 17]. This belief was later discredited when it was observed that radiolabelled serum proteins or other substances passed readily into the interstitium and equilibrated between blood plasma and testicular lymph, respectively [18, 19]. However, this equilibration took an appreciable time making testis vascular permeability intermediate between brain vessels and other endocrine glands [12, 20, 21]. Thus, rather than saying that the testis barrier does not exist at the level of endothelial junctions in the capillary wall, it would be more accurate to state that the impermeable or tight barrier does not exist in the testicular capillaries. On the same note, Plöen and Setchell, stated that “they use the term ‘blood-testis-barriers’ deliberately because they believe from the beginning that there was, and still is, evidence of more than one barrier” [22].

Complete exclusion of dyes and radiolabelled substances from the seminiferous tubules led to the assumption that there is a more complete/absolute barrier in or around the seminiferous tubules [14, 15, 20]. The SCs lying on the basal lamina of the seminiferous epithelium exhibit specialized inter-SC junctions. These junctions involve a significant narrowing of the intercellular space between adjacent SCs, termed as “close junctions” and a complete obliteration of this interspace by fusion of cell membrane between adjacent SCs defined as “tight junctions”. The tight junctions between adjacent SCs are an integral part of the BTB/SC barrier as they completely restrict the passage of small tracers (ferritin, peroxidase and lanthum) across the seminiferous epithelium, thus constituting an effective impermeable BTB/SC barrier [2]. In addition to tight junctions, the basal ectoplasmic specializations, gap junctions and desmosomes also contribute to the functional BTB/SC

barrier (extensively reviewed in [23, 24]). In rodents but not in large mammals, peritubular myoid cells surrounding the seminiferous tubules also create a semi-permeable barrier by restricting the passage of large electron-opaque particulates (carbon and thorium dioxide) and limiting the entrance of small molecules (lanthum) in 85-90% of the tubules [2, 25]. Thus, the endothelial and myoid cells contribute to the testis barrier nevertheless tight junctions between adjacent SCs form an effective impermeable barrier.

4. Blood-Testis-Barrier/Sertoli Cell Barrier and its Role in Germ Cell Immune Protection

Central tolerance, education of the immune system to discriminate between self and non-self antigens, is essential to generate a robust and effective immune response capable of clearing foreign pathogens without inducing autoimmunity. Central tolerance is achieved prior to birth by presenting tissue specific self-antigens to maturing lymphocytes. During this process, lymphocytes expressing high affinity for self-peptide/major histocompatibility complex (MHC) undergo negative selection and are eliminated by apoptosis [26]. At birth spermatogonia are the only germ cells present in the testis, with the spermatocytes and spermatids appearing around puberty [27]. Studies were designed to characterize the temporal expression of new cell surface auto-antigens during spermatogenesis by immunizing rodents with syngeneic germ cells, testicular lysate or whole semen. Immune sera was then collected from these animals and analysis of its reactivity to specific testicular germ cells revealed that the auto-antigens are expressed by meiotic and post-meiotic germ cells, but not by spermatogonia and somatic cells [5, 6, 28]. Given that these advanced germ cells appear after the immune system is educated, they are highly immunogenic [5, 6, 28]. For instance, immunization of rodents with syngeneic testicular homogenate (with or without adjuvant) induced experimental autoimmune orchitis, characterized by antibody deposition, infiltration of leukocytes around the rete testis, tubuli recti (section connecting seminiferous tubule with the rete testis) and peripheral seminiferous tubules and eventually germ cell loss [28, 29]. The response was antigen specific as no inflammation was observed in other organs and liver homogenate did not induce an inflammatory response.

The BTB/SC barrier develops shortly before the appearance of pachytene spermatocytes and divides the seminiferous tubule into two compartments-the basal compartment, containing spermatogonia and preleptotene spermatocytes and the adluminal compartment containing all subsequent stages of spermatocytes, as well as spermatids and spermatozoa (Fig. 1a) [2, 5]. Despite ample evidence of the existence of the BTB/SC barrier, its significance was largely overlooked until it was noticed that this barrier is not present in the neonatal rat testis but develops as rats attain puberty (around 15 days of age) [14]. To investigate the importance of the BTB/SC barrier, Setchell et al., studied the rate of passage of various radiolabelled substances from blood plasma into the testis interstitium and the seminiferous tubules by collecting testicular lymph and rete testis fluid, respectively [21]. In comparison with plasma and testicular lymph, rete testis fluid contained high concentrations of potassium, chloride and amino acids and low concentrations of sodium, bicarbonate and proteins [21]. Additionally, the immunoglobulin concentration in the rete testis fluid was approximately 0.2% the concentration in serum [30]. Later, fluid collected directly from the

seminiferous tubules revealed that the amount of immunoglobulin was even lower, as it was undetectable in an assay that compared the levels of immunoglobulin in seminiferous tubule fluid with those in rete testis fluid [31]. Under normal conditions, immune cells including macrophages, T cells and dendritic cells were present in the testis interstitium. However, their presence was not detected beyond the myoid cells and thus they are excluded from both basal and adluminal compartments of the seminiferous tubules (reviewed in [7, 32]). Based on these observations, it was suggested that the BTB/SC barrier sequesters the germ cells and prevents the immune cells from gaining access to these developing germ cells.

An example of the immunological importance of the BTB/SC barrier in spermatogenesis was demonstrated in SC specific androgen receptor knock out mice (Ar-KO) [33]. In these mice, biotin was detected in the adluminal compartment indicating increased permeability of the BTB/SC barrier to small molecules. Additionally, antibody deposition on advanced germ cells along with increased infiltration of plasma B cells within the seminiferous tubules was observed. Ultimately, spermatid differentiation was severely altered in these mice and germ cells were lost, suggesting that in normal testis the BTB/SC barrier effectively prevented the generation of humoral immune response against advanced germ cells. The spinal cord injury (SCI) model further highlights the role of BTB/SC barrier in barring antibodies and immune cells from infiltrating the adluminal compartment of the normal testis. The inflammatory response after SCI has been associated with tight junction disruption within the uroepithelium of the bladder. In an attempt to investigate whether the higher incidence of male infertility following traumatic SCI could be attributed to disruption of the BTB/SC barrier caused by SCI-associated inflammatory response, the integrity of the BTB/SC barrier and autoimmune response after SCI in rats was explored [34]. Increased permeability of the BTB/SC barrier to the small molecular weight molecule, gadopentate dimeglumine, following acute (72hrs) and chronic (10 month) SCI in adult rats indicated that the integrity of the BTB/SC barrier was breached. Furthermore, increased antibody deposition, immune cell (mast cells and macrophages) infiltration within the testicular interstitium and seminiferous tubules and germ cell apoptosis in SCI rat testes was observed [34]. Collectively, the BTB/SC barrier creates a conducive environment necessary for completion of spermatogenesis and sequestering the auto-immunogenic germ cells from the immune system.

4.1. Blood-Testis-Barrier/Sertoli cell barrier- not the sole criteria for immunological protection of germ cells

Despite the above-mentioned evidence favoring the critical immunological aspect of the BTB/SC barrier in spermatogenesis, it being the sole criteria was questioned by several studies. For instance, not all the germ cells with auto-immunogenic antigens are sequestered behind the BTB/SC barrier as immunization of mice with syngeneic testicular homogenate led to IgG antibody deposition in the basal compartment of 30-40% of seminiferous tubules (Fig. 1b and c) [28]. Moreover, antibodies present in the orchidectomized mouse sera reacted with germ cells isolated from prepubertal mice along with pubertal and adult mice. The prepubertal mouse (9-10 days) testis contains preleptotene spermatocytes, which are normally located in the basal compartment of the seminiferous tubules [28]. Adoptive transfer of activated CD4 T cells isolated from rodents with orchitis successfully transferred

the disease to normal syngeneic recipients with intact BTB/SC barrier [29, 35]. This further suggests the presence of germ cell auto-antigens, outside the intact BTB/SC barrier, which are accessible to immune cells that induce orchitis.

In most non-mammalian vertebrates (fish and amphibians), the effective BTB/SC barrier appears after the completion of meiosis when haploid germ cells are present [36], suggesting that the BTB/SC barrier is not solely responsible for successful progression of spermatogenesis. In zebrafish the effective BTB/SC barrier is formed during meiosis I as evident by the exclusion of small molecular weight tracer (lanthum) from the lumen of spermatogenic tubules [37]. However, lanthum was still able to penetrate around the leptotene/zygotene spermatocytes indicating these auto-antigenic germ cells are accessible to immune cells. Additionally, in mink, a seasonal breeder, the BTB/SC barrier is cyclically disrupted during the nonbreeding period and the development of auto-immunogenic spermatocytes is possible in the absence of a complete, impermeable BTB [38]. Maturation of these auto-antigenic germ cells without an effective BTB/SC barrier further indicates that other factors are involved in protecting testicular germ cells. Other evidence that immune protection of maturing germ cells requires more than just the BTB/SC barrier comes from the tight junction protein claudin 11 knockout mice [39]. Regardless of disrupted SC tight junctions, autoantibody production in the sera or its deposition within the seminiferous tubules was not observed in these knockout mice. Consistent with the absence of autoantibodies, no CD4 T cell infiltration was detected in the testes [39]. Similarly, administration of a mutant occludin peptide in adult rats breached the integrity of the BTB/SC barrier as shown by presence of fluorescent molecular probe (FITC-inulin) within the seminiferous tubules. Despite germ cell loss, an autoimmune reaction against testis-antigens was not generated [40]. Altogether, in normal testis, sequestration of most germ cells behind the BTB/SC barrier prevents immune cells from gaining access to these 'new antigens', however it is not the only criteria for protecting germ cells.

5. Nonsequestered Auto-antigenic Germ Cells- how are they Protected?

The presence of auto-antigenic germ cells outside the BTB/SC barrier raises the question that despite their easy access by the immune system, why is an immune response generally not generated against these germ cells. Induction of peripheral tolerance against these antigens and the presence of immunoregulatory factors in the testis, making the whole testicular environment immune privileged rather than just the adluminal compartment created by the BTB/SC barrier, could explain this phenomenon.

5.1. Induction of peripheral tolerance

Although central tolerance effectively eliminates highly auto-reactive lymphocytes, it spares cells that react weakly to self-antigens. Furthermore, not all self-antigens are expressed at the primary site of lymphocyte development, the thymus. Thus, other mechanisms must exist to supplement central tolerance [26]. Peripheral tolerance, involving regulatory T cells (Tregs) and immature/tolerogenic dendritic cells (DCs), is one mechanism that prevents an immune response to self-antigens [41, 42]. The testis interstitium contains DCs that are fully equipped to activate T cells under inflammatory conditions [43], yet DCs isolated from normal testis and testicular draining lymph nodes are unable to induce T cell proliferation

[32]. Antigen presentation by these tolerogenic DCs induces Tregs rather than auto-reactive T cells. The presence of Tregs in the rodent testis interstitium and/or testis draining lymph nodes have been reported and it has been shown that Tregs isolated from the testicular draining lymph nodes, from both normal and orchitis animals, were able to inhibit proliferation of auto-reactive T cells in response to testicular antigens [32]. Immunization of male and female mice with LDHc4 (testis specific isoform of Lactate Dehydrogenase) generated an antibody response of considerably greater magnitude in female than in male mice, although they responded equally to ovalbumin [44]. Thus, demonstrating that in male mice Tregs or other tolerogenic immune cells, specific to testicular antigens, dampened the immune response against exogenously injected LDHc4. Tung proposed a “selective” antigen sequestration model to highlight the immunological protection provided by peripheral tolerance along with the BTB/SC barrier to the testicular germ cells. He suggested that the peripheral tolerance protects the nonsequestered germ cells while the BTB/SC barrier protects the more advanced germ cells.

Besides nonsequestered antigens, peripheral tolerance can also be induced against spermatozoa. For instance, in vasectomized mice, sperm antigens coming from the inflamed epididymis were recognized by the immune cells, yet autoantibodies were not detected in these animals for as long as 6 months [35]. Moreover, unilaterally vasectomized mice were highly resistance to orchitis induced by testis-antigen immunization. Successful induction of experimental autoimmune encephalomyelitis in these mice suggested that this tolerant state was testis antigen-specific. Treg depletion in unilaterally vasectomized mice led to an autoantibody response against sperm antigens, lymphocyte infiltration within the seminiferous tubules and germ cell loss, suggesting Tregs have the capability to foster a tolerogenic state to germ cells in the testis [35]. Additional evidence demonstrating induction of peripheral tolerance against post-meiotic germ cells came from the study where transgenic ovalbumin was expressed in the elongated spermatids at high and low concentrations in two BALB/c mouse lines ([45], also discussed in [46]). High but not low expression of ovalbumin led to detection of ovalbumin in residual bodies and in immune complexes within the interstitium. Active immunization with exogenous ovalbumin failed to induce orchitis in mice expressing high concentration of ovalbumin, suggesting induction of tolerance against this antigen. This challenges the prevailing dogma that advanced germ cell antigens are completely sequestered behind the BTB/SC barrier and are never presented to the immune cells.

5.2. Entire testis is immune privileged rather than just the adluminal compartment

Peripheral tolerance induction by immature DCs and Tregs explains why under normal conditions an immune response is not generated against auto-antigenic germ cells present outside the BTB/SC barrier, but it also brings up another question, why are testicular DCs different from DCs present in other non-immune privileged tissues or why are they tolerogenic? Besides DCs, rodent testicular macrophages also exhibit regulatory properties as they poorly stimulate T cell proliferation *in vitro* and have a reduced capacity to produce pro-inflammatory cytokines compared to other non-immune privileged tissue macrophages (reviewed in [47]). The local microenvironment, predominant in immunoregulatory factors, seems to play an important role in inducing tolerogenic DCs and macrophages. For instance,

DCs generated *in vitro*, in the presence of immunoregulatory factors such as interleukin (IL)-10, transforming growth factor (TGF)- β , indoleamine 2-3-dioxygenase (IDO) and/or prostaglandinE2 exhibit tolerogenic properties [48, 49].

Immunosuppressive agents present in testicular fluid have been reported previously. For instance, fluid collected from the interstitium and seminiferous tubules (maximal at stages II-VIII) exhibits immunosuppressive activity as shown by a reduced proliferation of lymphocytes [50, 51]. Thus, the entire testis is immunoprotective rather than just the adluminal compartment of the seminiferous tubules, which explains the survival of auto-antigenic germ cells outside the BTB/SC barrier. The immune privileged status of the testis interstitium is supported by several studies. To begin with, traumatic needle injury induced formation of spermatocytic granulomas (mass of degenerating germ cells surrounded by immune cells) in the epididymis but not in the testis interstitium, although extravasation of germ cells occurred in both organs [52]. Allogeneic or xenogeneic tissue (skin fragments, islets or parathyroid tissue), transplanted within the testis interstitium (outside the BTB/SC barrier) enjoyed prolonged survival as compared to tissue transplanted to non-immune privileged sites (reviewed in [8, 53]). Additionally, allogeneic germ cells have been transplanted into the testes of farm animals without the use of immune suppression [54-58]. When germ cells were injected into the seminiferous tubules via the rete testis, this led to generation of live offspring in several of these studies [56, 58]. Detection of the transplanted testicular cells within the basal compartment of the seminiferous tubules [54-58] without generation of an immune response provides further evidence that the local testicular environment containing immunoregulatory factors along with the BTB/SC barrier is required for protecting auto-antigenic germ cells.

6. Immune Protection by Sertoli Cells

Immune privilege in the testis, including tolerogenic immune cells, is an active phenomena governed by locally produced immunoregulatory factors. Although, the imperative role of other testicular somatic cells (peritubular myoid cells and Leydig cells) in testis immune privilege cannot be underestimated (reviewed in [7]), here we will focus on SCs. Evidence for SCs as key regulators in testis immune privilege comes from studies where SCs provided protection to co-grafted non-testicular cells when transplanted across immunological barriers (allo- or xeno-transplantation) (reviewed in [8]). The importance of SCs in protecting auto-antigenic germ cells is best exemplified by transplantation studies using non-obese diabetic (NOD) mice, an autoimmune type 1 diabetes model, as recipients. NOD mice develop auto-reactive T cells and antibodies, which can reject transplanted syngeneic islets. Co-transplantation of SCs with syngeneic islets underneath the kidney capsule of NOD mice significantly prolonged islet survival compared to islet only controls [59, 60]. Additionally, 40-64% of the transplanted NOD mice remained normoglycemic for at least 60 days whereas none of the islet only recipients remained normoglycemic beyond 14 days after transplantation [59, 60]. The protection provided by SCs was not limited to syngeneic pancreatic islets as SCs also prolonged the survival of allogeneic or xenogeneic islets, adrenal chromaffin cells, xenogeneic hepatocytes, xenogeneic neurons, allogeneic or xenogeneic skin and heart grafts (reviewed in [8]). This protection was likely due to the modulation of the immune response and not the physical BTB/SC barrier as most of the co-

transplanted cells were located near, but not surrounded by, the SCs at the graft site [8, 61, 62]. Additionally, in NOD mice, SCs transplanted underneath the right kidney capsule provided protection to syngeneic islets transplanted to the contralateral kidney [59]. Collectively, this demonstrates that rather than just serving as a physical wall (by creating the BTB/SC barrier) between leukocytes and antigens (germ cells in testis and transplanted cells at ectopic sites) SCs have the ability to modify the immune response to these antigens.

7. Immune Modulation/Tolerance by the Sertoli Cells

When one thinks of testicular immune tolerance, attention is automatically drawn to the tolerogenic antigen presenting cells (APCs) and their role in inducing Tregs. Although, the role of tolerogenic APCs in inducing Tregs is indisputable, it is logical that there is a key regulator present in the testis that causes these immune cells to deviate from their normal course of development and turn into tolerogenic APCs and/or has the ability to directly induce Tregs. Of all the somatic testicular cellular components, SCs best fit the description of a key regulator. Strong evidence that SCs have the capability to induce regulatory immune cells comes from transplantation studies. For instance, intraperitoneal transplantation of encapsulated porcine SCs into NOD mice led to diabetes prevention and reversion in 88 and 81% of the recipients, respectively. Transplanted SCs generated an anti-inflammatory cytokine milieu (both locally and systemically) which skewed the immune response from destructive to protective as evident by increased Treg number and decreased IL-17 producing CD4 T cells [63]. In another study, the protection provided by SCs to syngeneic islets transplanted in NOD mice was attributed to TGF- β production by SCs, as administration of anti-TGF- β antibody abrogated the protection, which modulated the immune response from destructive (interferon (IFN)- γ producing cells) to protective (IL-4 producing cells) [59]. Additionally, in our transplantation model, where SCs were transplanted as allografts underneath the kidney capsule of naïve BALB/c mice, an increase in Tregs in SC grafts compared to rejected mouse SC line grafts was observed (Kaur G and Dufour JM, unpublished data). Thus, further demonstrating that SCs have the capability to modify the immune response when transplanted to ectopic sites. Based on these studies, it is plausible that SCs modulate the immune response in the testis.

The exact mechanism(s) by which SCs induce Tregs (within or outside the testis) are not known. Additionally, whether induction is direct and/or indirect (involves APCs) makes the question more complicated. So far, only one study addressed the Treg induction mechanism by SCs [64]. In this study, SCs were treated with IFN- γ which induced the expression of B7-H1 (ligand for negative regulatory receptor) and MHC-II (usually present on professional APCs) by SCs. SCs were then co-cultured with purified syngeneic T cells which resulted in an increase in CD4 Tregs (31.8%) compared to controls (T cells cultured alone, 4.7%) [64]. This suggests the SCs were acting as tolerogenic APCs, as they express B7-H1 and MHC-II but lack positive co-stimulatory molecules, and thereby have the ability to induce Tregs directly. In testis, the semi-permeable barrier created by peritubular myoid cells excludes the T cells from gaining access to SCs. However, the presence of light-staining cells (resembling mononuclear cells morphologically) in the myoid cell layer has been documented in the rat seminiferous tubules [65] leading to the speculation that in testis SCs could also be involved in presenting germ cell antigens to T cells [64]. As mentioned earlier

antigen presentation by tolerogenic APCs induce Tregs rather than auto-reactive T cells, thus direct induction of Tregs by SCs in the testis is possible. Furthermore, rather than inducing Tregs directly, SCs could induce tolerogenic APCs in the testis interstitium, by secreting immunoregulatory factors, which then induce Tregs in the interstitium or in draining lymph node (Fig. 2).

Although, the Treg induction mechanism is obscure, characterization of immunomodulatory factors expressed by SCs, which are involved in inducing tolerogenic immune cells, are well documented. For instance, SCs produce TGF- β and IDO, which are involved in inducing Tregs and tolerogenic DCs [63, 66]. TGF- β and IDO have been implicated in SC protection of transplanted islets or reversal of diabetes, respectively, in NOD mice [59, 63]. Furthermore, galectin-1, a highly conserved β -galactosidase-binding protein, was also detected in SCs [67, 68]. Galectin-1 inhibits pro-inflammatory cytokine secretion and induces the production of CD4 Tregs [69]. Furthermore, *in vitro* treatment of macrophages with immunoregulatory factors (TGF- β and IL-10) resulted in the production of regulatory macrophages, which suppressed T cell proliferation and induced Treg production [70]. Additionally, activin A production by SCs and its involvement in inducing alternatively activated testicular macrophages has been reported [71]. Alternatively activated macrophages are involved in wound repair, tissue remodeling, reducing inflammation and modulating the immune response [72]. These are just few examples of immunoregulatory factors expressed by SCs. From our microarray analyses data we know that SCs are capable of producing a wide variety of immunoregulatory factors, which could be impacting the immune system [73]. Nonetheless, their exact role in inducing tolerogenic immune cells needs further investigation. In general, immunoregulatory factors expressed by SCs create a tolerogenic environment in the testis (involving Tregs and tolerogenic APCs), explaining the survival of auto-antigenic germ cells present outside the BTB/SC barrier.

However, if testicular immune tolerance is breached SCs also have back up mechanism(s) to inhibit humoral and cell-mediated immune responses. For instance, SCs can inhibit the proliferation of NK, B and T cells (Fig. 3a) [74-77]. SCs also express or secrete several complement inhibitors [78-80], which could prevent complement mediated cell lysis (Fig. 3b). Recently, through microarray analyses and qRT-PCR we have demonstrated for the first time that SCs express serine protease inhibitor (SERPIN)G1 [73]. SERPING1 targets the initial step of complement cascade activation i.e. C1 complex thereby preventing the formation of C3 convertase (Fig. 3b) [73]. SCs also express or secrete apoptosis inhibitors such as serpin3n and protease inhibitor-9, which can inhibit NK and T cell-mediated death (Fig. 3a) [81-83]. Although, SCs are equipped to suppress the immune response, in normal testis it is likely that instead of constantly suppressing the immune response SCs induce immune tolerance against testicular germ cells.

In conclusion, immune privileged SCs protect the majority of the auto-antigenic germ cells by sequestering them behind the physical BTB/SC barrier. Besides, preventing the immune cells from directly gaining access to these advanced meiotic and post-meiotic germ cells, SCs could also induce tolerance to these germ cells by presenting their antigens in a controlled manner. Furthermore, the survival of nonsequestered auto-antigenic germ cells depends on the local tolerogenic testicular environment (including regulatory immune cells)

created by the immunoregulatory factors expressed by SCs (along with other somatic testicular cells).

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Highlights

- Appearance of germ cells, expressing novel cell surface and intracellular proteins, after induction of the systemic tolerance makes them auto-immunogenic.
- Sertoli cells (SCs) protect germ cells by forming the Blood-Testis-Barrier (BTB)/SC barrier, includes the tight junctions between Sertoli cells along with the body of the SCs, and modulating the local environment of the testis.
- The BTB/SC barrier sequesters the majority of auto-antigenic germ cells and prevents the immune cells from gaining access to these developing germ cells.
- Immunomodulatory factors expressed by SCs protect the nonsequestered auto-antigenic germ cells by inducing regulatory cells either directly and/or indirectly.

Figure 1.

A) Localization of the BTB/SC barrier and cellular components of the testis. Testis interstitium consists of Leydig cells, macrophages (MΦ), tolerogenic DCs, T cells and blood vessels. The seminiferous tubules are composed of SCs and maturing germ cells surrounded by peritubular myoid cells. Tight junctions between adjacent SCs along with the body of the SCs form the BTB/SC barrier, which divides the tubules into the basal and adluminal compartments. B) Autoimmune orchitis. At the onset of orchitis, antibodies deposit on preleptotene spermatocytes present in the basal compartment of the tubules. Auto-reactive immune cells (cytotoxic MΦ and T cells) infiltrate the interstitium. C) Eventually, the disrupted BTB/SC barrier along with increased infiltration of auto-reactive immune cells results in arrest of spermatogenesis and germ cell loss. Y, auto-antibody.

Figure 2. Immune modulation by SCs

Immunoregulatory factors (such as TGF- β , IDO, galectin-1, activin A) expressed/secreted by SCs modulate the immune response to maintain a tolerogenic environment either directly, by inducing Tregs, or indirectly by inducing tolerogenic APCs (regulatory M Φ or tolerogenic DCs). These tolerogenic APCs present antigens to T cells either in the interstitium or testes draining lymph nodes and convert them into Tregs. Blue triangles, immunoregulatory factors.

Figure 3. Immune suppression by SCs

A) SCs inhibit proliferation of NK, B and T cells by expressing/secreting immunosuppressive factors. SCs also inhibit IL-2 production by T cells resulting in reduced proliferation. SCs express several apoptosis and complement inhibitors to prevent NK and T cell-mediated death, and complement-mediated lysis, respectively. Blue triangles, immune suppressive factors. B) Antigen-antibody complexes interact with C1q and result in formation of C1 complex, which then further activates the complement cascade by generating C3 convertase. C3 convertase recruits C3 and forms the C5 convertase. C5

convertase culminates in formation of the membrane attack complex (MAC) after recruiting other factors (C5b-C9). Formation of MAC results in compliment-mediated cell lysis. SCs inhibit activation of the complement cascade by expressing/secreting inhibitors (red boxes) which prevents the C1 complex, C3 convertase, C5 convertase and MAC formation.