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REVIEW

Somatic-Immune Cells Crosstalk In-The-Making of Testicular Immune Privilege

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Abstract

Immunological infertility contributes significantly to the etiology of idiopathic male infertility. Shielding the spermatogenic cells from systemic immune responses is fundamental to secure normal production of spermatozoa. The body's immune system is tuned with the host self-components since the early postnatal period, while sperm first develops during puberty, thus rendering spermatogenic proteins as 'non-self' or 'antigenic.' Development of antibodies to these antigens elicits autoimmune responses affecting sperm motility, functions, and fertility. Therefore, the testes need to establish a specialized immune-privileged microenvironment to protect the allogenic germ cells by orchestration of various testicular cells and resident immune cells. This is achieved through sequestration of antigenic germ cells by blood–testis barrier and actions of various endocrine, paracrine, immune-suppressive, and immunomodulatory mechanisms. The various mechanisms are very complex and need conceptual integration to disclose the exact physiological scenario, and to facilitate detection and management of immunogenic infertility caused by disruption of testicular immune regulation. The present review aims to (a) discuss the components of testicular immune privilege; (b) explain testicular somatic and immune cell interactions in establishing and maintaining the testicular immune micro-environment; and (c) illustrate the integration of multiple mechanisms involved in the control of immune privilege of the testis.

Keywords Blood-testis barrier · Immunosuppression · Spermatogenesis · Sperm auto-antigens- · Testicular immune privilege

Introduction

Infertility affects 48.5 million couples globally, in which 20–30% of infertility is associated with male factors alone [1, 2]. A detailed analysis showed that 2.5 to 12% of men are infertile with Africa and Central/Eastern Europe having the highest rates of infertility [1] but the high prevalence of male infertility makes it a global concern.

The complex dichotomy of testicular somatic and immune cells defines the complex etiology of male infertility [3]. The unique anatomical structure and functional immune privilege allow the testes to bear a specialized immune microenvironment [4]. This specialized immune

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microenvironment enables the testes to tolerate the antigenic proteins expressed by the spermatozoa, thereby also protecting these allogenic sperm cells from systemic immune attack [5]. The testes house two main groups of cells that play a crucial role in the maintenance of immune homeostasis: somatic cells and testicular immune cells [6]. Spermatogenic cells need to be protected from systemic immune responses or in other words, prevention from auto-antigens to induce autoimmune responses is fundamental to male fertility [3]. These features create the testicular 'immune privilege' and provide immune tolerance to various allografts. Maintenance of testicular immune privilege requires several specialized levels of immune regulations. Disrupted testicular immune homeostasis during pathological conditions, such as infections, inflammation, or congenital issues, may trigger autoimmunity and immunogenic infertility [7].

Several mechanisms related to testicular immune privilege have been unveiled over the last few years but we are still far from having full knowledge of those processes and having enough information to integrate the different mechanisms [8, 9]. The mechanism of testicular immune privilege may be clearly perceived with a proper concept on systemic and testicular immune tolerance and the individual role that testicular somatic cells, immune cells, and immunomodulatory molecules play in maintaining this immune privilege. The major aims of this work are to discuss the components of testicular immune privilege, review testicular somatic and immune cell interactions during the assembly and maintenance of testicular immune micro-environment, and to integrate the multiple mechanisms involved in the control of testicular immune privilege.

Definition of Immune Privilege in the Testis

Some sites in the human body have immunological privilege, i.e., they may tolerate foreign antigens without causing an inflammatory immune response [10]. Immune privilege is considered an evolutionary adaptation to prevent an inflammatory response to infections from damaging essential tissues [10]. Previously, antigens in immune-privileged locations were considered to have been physically hidden from the immune system and thus disregarded. Later investigations, however, have shown that antigens that leave the sites of immunological privilege may generate immune responses to these antigens and that the cells of immune effectors can have access to immunologically privileged locations. Now, it is obvious that an active rather than a passive mechanism maintains the immune privilege [11].

During the pubertal period, immune privilege within the testis develops in order to sequester antigens produced by germ cells for spermatogenesis via the blood testis barrier (BTB) and through specialized mechanisms performed by immune cells [4]. The BTB provides a physical barrier for the adluminal part of the germinal epithelium from antigens that could induce autoimmune responses but not all germ cell autoantigens are controlled by the BTB [4]. The release of growth factors and anti-inflammatory cytokines allow the BTB to develop immunosuppressive privilege. Along with the tolerogenic microenvironment established by Sertoli cells, tolerogenic dendritic cells and regulatory T cells (Tregs) are responsible for the establishment of the immunosuppressive microenvironment needed for immune privilege within the testis [4, 12–14]. The prohibition of autoimmune responses in the testis is due to local and systemic responses by tissue-specific cells and immune cells allowing for germ cells to express autoantigens to induce spermiogenesis [15, 16]. These mechanisms allow the development of tolerogenic functionality within the testis.

Anatomical Basis of Testicular Immune Privilege

The structure of the testis protects the processes of spermatogenesis and steroidogenesis — the two main testicular functions. To perform those duties, the anatomical and structural organization of the testis is very well defined. Indeed, the anatomical basis of testicular immune privilege is defined by suborganization of cells within the testis [4]. The seminiferous tubules and interstitial space are two discrete regions of the testis which are divided by the BTB [4, 14, 17], and that organization is pivotal for the immune privilege that is established in the testis.

Structural Organization of Seminiferous Tubules and Interstitial Space

The testis is histologically and functionally segregated, with androgen synthesis and spermatogenesis occurring in separate compartments. Androgens are synthesized in the Leydig cells in the interstitial compartment, interspersed between the tubules, whereas spermatogenesis takes place in the seminiferous tubules or germinal compartment [6, 13, 15]. The true septa divide the testis into lobules in the human infant and the septa extend from the fibrous capsule (tunica albuginea) that surrounds the testis [18]. These lobuli are less prominent in adult human testes, and entirely disappear in rodents [18]. The densely coiled seminiferous tubules that originate and end at the rete testis, and house the germinal compartment of the testis [9]. Myoid peritubular tissues, surrounding each tubule, provide structural support and generate peristaltic waves with their contractile elements [19]. Peristaltic movements aid the transfer of immotile spermatozoa through the seminiferous tubule, the rete testis, and henceforth through the epididymis [19]. However,

the peritubular cells do not produce a tight diffusion barrier, instead these cells and the Sertoli cells express an array of cytokines, growth factors, and components of the seminiferous tubular basal membrane [20, 21]. The Sertoli cells are the main structural component of the seminiferous epithelium, which extend from basal lamina towards the tubular lumen and provide physical support to the developing germ cells besides providing them with the required nutrition and growth factors [15].

The testicular interstitial space is scattered with Leydig cells that synthesize testosterone via steroidogenesis. The interstitium also houses immune cells — lymphocytes, macrophages, dendritic cells, and mast cells — that protect the testis from blood borne pathogens [15] (Fig. 1).

Blood-Testis Barrier

Tight junctions (TJs), gap junctions, desmosome-like junctions, and basal ectoplasmic specializations between two neighboring Sertoli cells form the blood-testis barrier (BTB) in the seminiferous tubules in order to isolate the developed germ cells from the blood. The main functions of the BTB can be subdivided into three categories — anatomical, physiological, and immunological — that interact to define a testicular immune environment. The anatomical barrier is based on the function of the junctions that restricts further developed (haploid) germ cells from reaching the blood [4]. The physiological barrier is based on transporters that establish the interstitial milieu while the immunological barrier refers to the limitation of the systemic immune responses and autoantigenic germ cells arrest. However, spermatozoa lack protection from autoimmune attacks once they leave the seminiferous tubules because the BTB is terminated at the rete testis [4, 22, 23].

The ability of the BTB to sequester antigen and antibodies has been used to define the testicular immune privilege until further evidence showed that preleptotene spermatocytes and spermatogonia were able to express antigenic molecules [4, 15]. Since the germ cells in these developmental stages are not sequestered by the BTB, their antigenicity is suppressed by establishing the testicular immune tolerogenic microenvironment by the integrated actions of interstitial testicular somatic and immune cells, that are explained in the later sections of the manuscript.

Junctional Dynamics of the BTB

Completion of spermatogenesis needs migration of spermatocytes of the preleptotene and leptotene phases, from the basal part of the seminiferous epithelium, crossing the BTB to the adluminal compartment. Thus, there is progress from the diploid germ cells (not protected by the BTB) to the haploid state which are segregated by the BTB so that these postmitotic germ cells are restricted by the BTB to come in contact with the immune system averting immune-rejection of the spermatogenic cells that develop after immune tolerance. This process requires disassembling and reassembling of the Sertoli-Sertoli junctions as well as Sertoli-germ cell

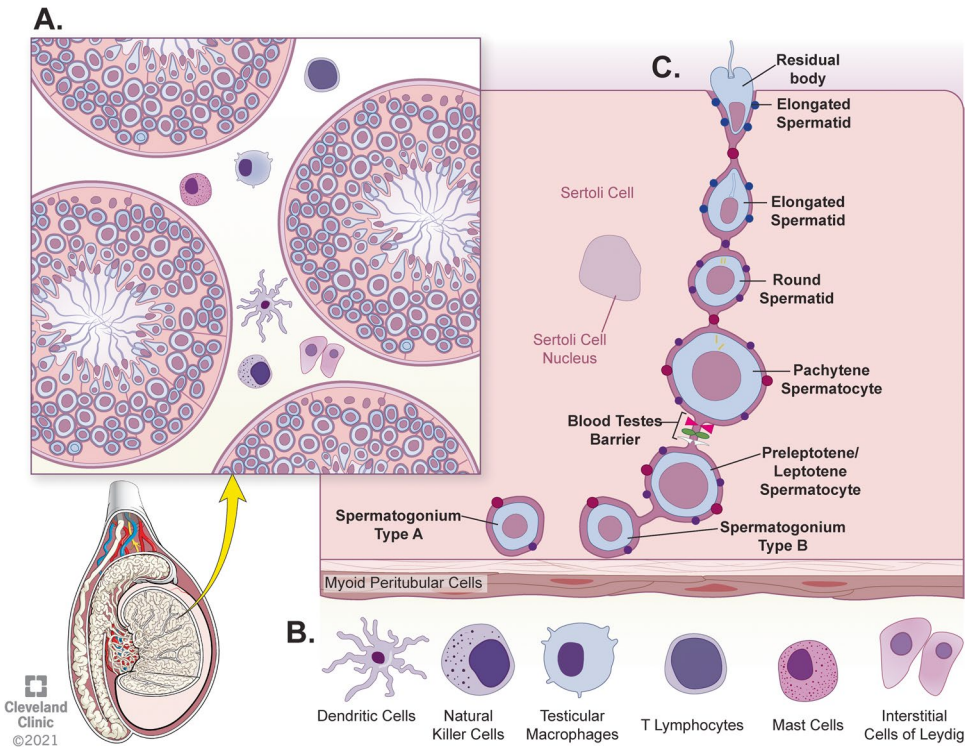

Fig. 1 Anatomical and cellular components of testicular immune privilege. (**A**) Seminiferous tubules and interstitial compartments. (**B**) Spermatogenic cells at different stages of development in association with Sertoli cells; The blood–testis barrier (BTB) is formed by tight junctions between adjacent Sertoli cells near the basement membrane; (**C**) Testicular interstitium showing Leydig cells and resident immune cells including macrophages and dendritic cells, T lymphocytes and mast cells

junctions [13]. The junctional dynamics of the BTB have been shown to be regulated by various cytokines, such as transforming growth factor-β3 (TGF-β3), interleukin 1-α (IL-1α), and tumor necrosis factor-α (TNF-α); endocrine and paracrine factors [24–26], growth factors [27] as well as nitric oxide (NO) [28]. The BTB is maintained by various adhesion protein complexes with the coordination of the N-cadherin/β-catenin of the gap junctions and occludin/ZO-1 of the tight junctions being most prominent [29]. These proteins are anchored in F-actin bundles and provide resistance to the barrier. Although the BTB goes through elaborate reorganization to permit preleptotene spermatocytes transit, the BTB-conferred immunological barrier is not compromised at any point of the epithelial cycle to protect the meiotic and post meiotic germ cells from any immune responses [30]. The minute regulations of the BTB dynamics are mainly mediated by the coordination of testosterone and transforming growth factor (TGF)-β2, that controls the BTB opening and closure, by differentially modulating the endocytosed integral membrane protein [31].

The BTB alone does not suffice the requirement of testicular immune privilege and restoration of this property needs integrated functions of immunoregulatory testicular somatic and immune cells.

Immune Cells in the Testicular Interstitial Space

Lymphatic vessels within the interstitial space of the testis allow access to afferent lymph nodes [14]. Studies have revealed that testicular interstitial tissue-organization is complex with an extensive "testicular lymphatic space" [32, 33]. The lymphatic space has continuous "peritubular lymphatic sinusoids" and the seminiferous tubule and interstitium have no cellular connections. Each seminiferous tubule is entirely surrounded by a sinusoid, and the near-by sinusoids freely communicate with each other via interstitial fenestrations. Thus, in case any cell or component need access to the seminiferous tubule, it must enter the lymph [32]. More recently, it was revealed that lymphatic capillaries are placed just beneath the tunica albuginea rather than in the interstitium between the seminiferous tubules [34]. In an experimental rodent model, normal lymphocytes administered into testes were seen to migrate between the seminiferous tubules followed by their drainage into the lymphatic vessels in the tunica albuginea [34]. Thus, even if the testis is an immune privilege site, the afferent lymph nodes are well-connected to it. Thus, to render protection against lymph borne antigens or pathogens, the testis bears most types of immune cells, including dendritic cells, macrophages, mast cells, and T lymphocytes in the interstitial space. In order not to affect the developing germ cells, these testicular immune cells, unlike their immune responsiveness in other sites, maintain a suppressed immunity in the testis [14]. The following section will discuss the role of the immune cells present in the testicular interstitial space, namely the lymphocytes, macrophages, dendritic cells, and mast cells in testicular immune homeostasis (Table 1).

Macrophages

Macrophages are the most common immune cell in the interstitial space of the testis and play a major role in the testicular immune modulation [5, 41, 48]. Macrophages can be broken down into two subgroups [5, 37, 48–50]. The

Table 1 Testicular immune regulation via anatomical barrier, testis-specific cells, and immune cells

Testicular components	Immune regulatory functions	References
Blood-testis barrier	Limits systemic immune responses and sequesters autoantigenic germ cells	[4, 11]
Leydig cells	Produce androgens that regulate expression of anti- and pro-inflammatory cytokines imposing immunosuppressive function	[35, 36]
Myoid Peritubular cells	Release cytokines: leukemia inhibitory factor, MCP-1, and TGPβ-2. MCP-1 cause inflammation by recruitment of leukocytes	[37]
Sertoli cells	Phagocytosis to remove residual bodies and apoptotic cells. Secrete immunosuppressive molecules to inhibit inflammatory responses of macrophages and T lymphocytes (T) in the interstitium	[11, 38]
Germ cells	In response to IFN- γ and INF- α, they secrete antiviral proteins. Express Fas ligand which can initiate apoptosis of lymphocytes	[39, 40]
Macrophages	Decreased expression of inflammatory factors in testis. Anti-inflammatory group express glycoprotein recognized by antibody ED2	[5, 33, 34, 41, 42]
Dendritic cells	Two important roles: lymphocytes activation by antigens and tolerate T cells to antigens via inhibition of autoimmune response	[13, 43]
Lymphocytes	Regulatory T cells (Tregs) play a major role in suppression of antigen specific immune response	[38, 44]
Mast cells	Induce collagen synthesis and proliferation of fibroblasts by secreting serine protease tryptase. Role of mast cells in testicular immune privilege remains unknown	[45–47]

dominating population of macrophages expresses a glycoprotein on the cells surface, CD163, which is recognized by an antibody, ED2 [51, 52]. These ED2-recognizing macrophages are classified as anti-inflammatory due to their upregulation of IL-10 and secretion of immunosuppressive factors [51]. The second population of macrophage expresses a lysosomal glycoprotein, CD68, which is recognized by an antibody, ED1 [48, 51]. ED1-recognizing macrophages are classified as pro-inflammatory due to their secretion of IFN-γ and TNF-α cytokines that induce an inflammatory response. Similarly, these macrophages may also activate T cells to express IFN-γ and TNF-α cytokines. Recent evidence shows a third subpopulation of macrophages that is recognized by both ED2 and ED1 antibodies [53]. These ED2 + ED1 recognizing macrophages have been shown to express nitric oxide synthase (NOS), which is not expressed by the ED2 recognizing macrophages while expressed in low levels by the ED1 recognizing macrophages [53]. Thus, to maintain the immune-suppressed environment in the testis, the anti-inflammatory ED2 + macrophages comprise the majority (80%); and the ED1 + macrophages comprise only 20% of testicular macrophages [37, 48].

Dendritic Cells

Derived from bone marrow, dendritic cells are specialized as antigen-presenting cells [15]. Dendritic cells play two important roles: lymphocytes activation by antigens and tolerate T cells to antigens via inhibition of autoimmune response. Dendritic cells minimize response to autoantigens while maximizing the response against foreign pathogens [4, 16]. It has been seen in murine model that majority of DCs in the normal testis express similar levels of MHC class II and co-stimulatory molecules (CD80 and CD86) as are expressed by DCs during inflammation [43]. But in normal testis, these DCs have been shown to express low levels of CCR7 mRNA and negative expression of IL-12p35 mRNA suggesting that the DCs are immature thereby preventing DCs-induced activation of T cells and rendering the DCs as tolerogenic [43, 54, 55].

Lymphocytes

Even under physiological conditions, lymphocytes are always present in the testicular interstitium [56]. The testicular lymphocytes mostly comprise of the T cells, predominantly the CD8 + T cells and less of CD4 + T cells, while in normal testis, no B cells can be found. The testicular lymphocyte population drastically increases during inflammatory conditions and in infertile men with sperm autoimmunity [38, 57] that suggest the roles of the lymphocytes in testicular adaptive immune responses. A study using rat testis showed that the testes possess immunoregulatory T cells, that include the CD4 + CD25 + regulatory T cells as well as the natural killer cells. Regulatory T cells functions to inhibit antigen-specific immune response [37, 44] as they are potent immunosuppressive cells that are significant players in establishing immune-tolerance [6, 49] and in vasectomy models, the regulatory T cells have been shown to regulate the balance between tolerogenic actions and autoimmune responses to sperm antigens [58]. Studies show that when allografts were placed in mice testes, the memory T cells were destroyed while the graft antigen-specific regulatory T cells were synthesized in order to sustain the tolerogenic environment in the testes [59–61]. These findings suggest that the regulatory T cells are the main lymphocytes contributing to immune privilege in the testis and whether the NK cells also have significant roles in testicular immune privilege are subjected to future research.

Mast cells

Mast cells regulate steroidogenesis in the male testis and high amounts of mast cells have been linked to male infertility [3, 4, 45, 46]. Mast cells induce the synthesis of collagen and the proliferation of fibroblasts by secreting serine protease tryptase [47, 62, 63]. These cells have been found to have impact upon regulatory T cells and immune-tolerance [35, 64]. One of the factors responsible for inflammation is the inactivation of regulatory T (Treg) cells, but the underlying mechanism is still unclear. It has been observed that mast cells, which are among the initial inflammatory mediators, can breach the regulatory T cells-mediated inhibition over effector T cells. Withdrawal of suppression of the regulatory T cells needs IL-6 derived from T cells and the OX40/OX40L axis. In an in vitro setup with IL-6 is in abundance and lack of Th1/Th2 cytokines, it had been shown that activated mast cells could skew regulatory T cells into IL-17–producing T cells thereby inducing inflammatory responses [35]. Contrary to the pro-inflammatory role of mast cells, it has also been reported that activated regulatory T cells may recruit mast cells via IL-9 in order to facilitate regional immune suppression [36, 64].

Tissue-Specific Cells of the Testicular Immunity

Tissue-specific cells — Sertoli Cells, myoid peritubular cells, and Leydig cells — play a pivotal role in the maintenance of testicular immune homeostasis through immunological functions [15] (Table 1).

Leydig Cells

The predominant cells located in the interstitial space are the Leydig cells which produce androgens and regulate spermiogenesis in the seminiferous tubules while also targeting organs outside the testis via peripheral circulation [65]. Androgens show immunosuppressive actions [66] by regulating the expression of anti- and pro-inflammatory cytokines produced by tissue-specific cells of the testis through mechanisms that will be later discussed [15]. It has been evidenced since long that Leydig cells can regulate the leukocyte number in the testis to restrict overt immune responses in the testes [56, 67].

Myoid Peritubular Cells

Multiple layers of myoid peritubular cells (MPCs) are myofibroblast-like cells that surround the seminiferous tubules in humans while in rodents they form a single layer. There are prominent evidences that MPCs can regulate testicular development, spermatogenesis, and Sertoli cell functions [68–70]. PModS is a major MPCs-derived factor that can regulate Sertoli cells functions, particularly the secretion of inhibin, transferrin, and androgen-binding protein [71, 72]. Other MCPs-derived mediators such as heregulins, IGF-I, and other cytokines are also essential in mediating the molecular interactions between MCPs and Sertoli cells [71].

Due to their location and structure, MCPs may have the ability to maintain the immune homeostasis of the testis. MPCs play role in testicular inflammation by releasing cytokines, leukemia inhibitory factor, MCP-1, and TGPβ-2. MCP-1 and thereby recruiting macrophages and/or inflammatory monocytes. In humans MCPs also produces TNF- α receptors 1 and 2 which recruits other inflammatory molecules [73].

Sertoli Cells

One of the main features of Sertoli cells is that they provide an immunoprotective environment in the testis. The immunoprotected environment is most likely established by secreted factors and molecules expressed on the surface of the Sertoli cells. This immunosuppressive property was shown in an experiment where allografts and xenograft were co-transplanted [74]. In this experiment, NOD murine Sertoli cells were extracted and implanted in NOD diabetic mice below the right renal capsule, and below the left renal capsule, the NOD islets were implanted. Among the mice with both the islets and Sertoli cells implants, majority (64%) were found to be normoglycemic at 60 days post-transplantation, while among the mice with only the islet grafts, none were normoglycemic. As observed via immunohistochemical analysis, Sertoli cell grafts in normoglycemic mice expressed high levels of transforming growth factor (TGF)-β1 and showed progressively decreased expression of FasL following the transplantation. Moreover, in these mice, the plasma TGF-β1 levels were also increased and when anti–TGF-β1 antibody were administered, the protection rendered by the Sertoli cells on islets survival were disrupted, while administration of FasL did not show any such disruptions. These observations revealed that the Sertoli cells, via the production of TGF- β1, can protect other cells from autoimmune attack, a property that is employed in normal testes to protect the developing germ cells.

Sertoli cells also use phagocytosis to remove residual bodies and apoptotic cells [75]. Most male germ cells undergo apoptosis during spermatogenesis, and the excess cytoplasm of the elongating spermatids are discarded as residual bodies. Sertoli cells must phagocytose all apoptotic germ cells and residual bodies in order to preserve testicular homeostasis [75]. Sertoli cell-mediated phagocytic activities assist spermatogenesis in many ways, including (1) decreasing the space scarcity for immense male germ cells to complete the differentiation process, (2) prohibiting noxious components produced by necrosis of apoptotic germ cells, (3) eliminating autoantigens that could cause an autoimmune response, and (4) recycling of the components of apoptotic germ cells and residual bodies as energy sources for Sertoli cells. The mechanism of phagocytosis by Sertoli cells involves several hypotheses. A universal mechanism by which phagocytes engulf apoptotic cells is by the association of class-B scavenger receptor type I (SR-BI) expressed on phagocytes and phosphatidylserine (PS) presented on apoptotic cell surfaces [75, 76]. Axl, Tyro3, and Mer (TAM) tyrosine kinase receptors, as well as growth arrest specific gene 6 (Gas6) which is their functional common ligand, are needed for Sertoli cells to effectively phagocytose apoptotic germ cells [75]. Besides the phagocytic activities, Sertoli cells also participate in testicular immunoprotection by inhibiting inflammatory responses of the T lymphocytes and macrophages in the testicular interstitium [3] (Fig. 2).

Germ Cells

Spermatogonia are susceptible to damage by inflammatory conditions as unlike the germ cells in meiotic and post-meiotic stages, spermatogonia are not segregated by the BTB from the testicular interstitial immune cells and their secretions. However, in vitro studies have shown that the spermatogonial immune responses towards infection are weak with even no expression of active interferons, which may be to maintain the tolerogenic property of the testes [39]. The early spermatids and spermatocytes at pachytene stages also show very weak immune response when counteracted with virus (as shown by using Sendai virus in in vitro set-up), but they express IFNs though in very

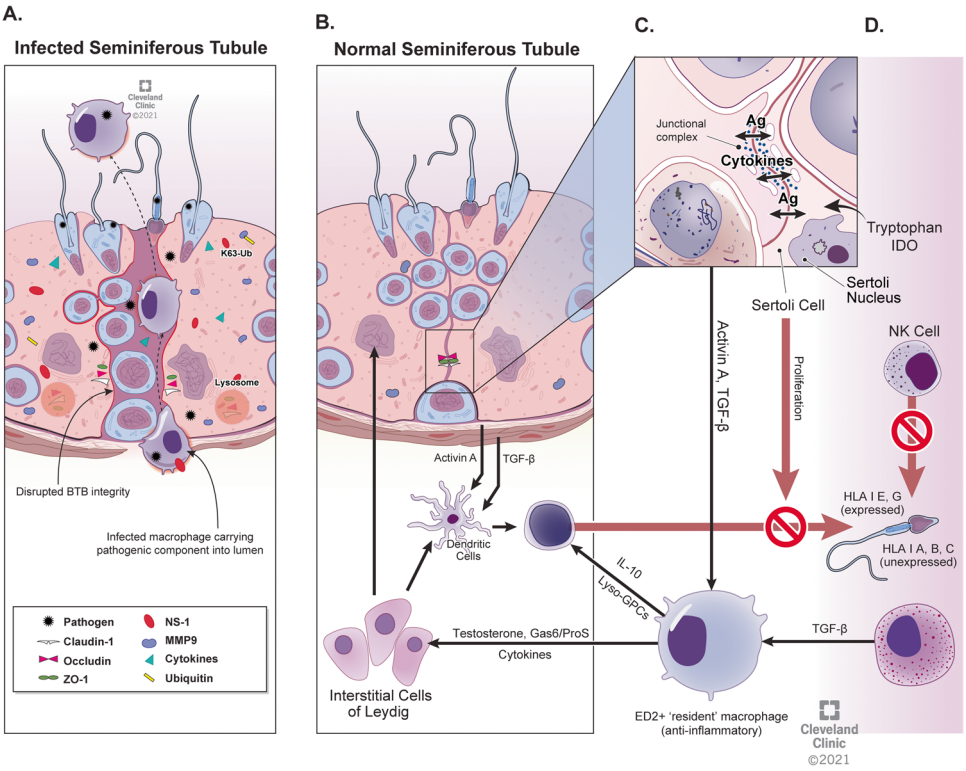

Fig. 2 Testicular infections-induced damage and testicular inter-cellular networking and immunoregulations in the establishment of immune privilege. (**A**) Schematic representation of the testicular infections-mediated immune responses that damages the blood-testes-barrier (BTB) and disrupts the seminiferous tubules affecting the entire germ line. NS-1, non-structural protein-1; MMP-9, matrix metalloprotease-9; ZO-1, Zonula occludens-1; (**B**) In normal uninfected seminiferous tubule, the BTB sequesters the developing spermatocytes (pre-leptotene/leptotene/mid-pachytene), specifically the primary and secondary spermatocytes bearing auto-antigens, from the interstitial space and thereby protecting the germ cells from local or systemic immune attack. Sertoli cells phagocytize the senescent germ cells, apoptotic germ cells, and sperm residual cytoplasm and thus present the sperm antigens to the testicular interstitium where the testis-specific immune cells are present. These immune cells comprise of resident macrophages, dendritic cells, T cells, and natural killer (NK) cells. The Leydig cells and Sertoli cells act to maintain the number and functions of the testicular macrophage. Testosterone modulates the functions of the macrophages and dendritic cells so that when sperm antigens are presented by these cells to the T cells, a tolerogenic (type 2) response is elicited. This mechanism needs the coordinated actions of immunoregulatory factors from the somatic and immune cells, mentionably, the transforming growth factor β (TGFβ) and activin A secreted by the Sertoli cells, anti-inflammatory cytokine, interleukin 10 (IL10) by the macrophages and dendritic cells; (**C**) In parallel to these processes, activated T cells are killed via the aid of Fas ligand (FasL), indoleamine 2,3 dioxygenase (IDO) or lyso-glycerophosphatidylcholines (lyso-GPCs); (**D**) Moreover, NK cells and CD8 + cytotoxic T cells fail to recognize the sperm antigens due to lack of expressions of classical HLA (Human Leukocyte Antigen) class I A, B and C. As a result, sperm antigen-specific adaptive immune responses are suppressed or subverted thereby preventing disruption to sperm development and sperm functions. Disruption of these mechanisms may lead to autoimmune responses against the sperm antigens (a type 1 response), followed by unregulated inflammatory reactions resulting in production of sperm antibody, induced germ cell apoptosis, compromised sperm functions, and eventually infertility

low levels [77]. However, the post-meiotic and meiotic germ cells express Fas ligand (FasL) and thus can contribute in limiting the number of lymphocytes by inducing apoptosis in these germ cells [40]. Thus, germ cells themselves do not exhibit adequate anti-viral responses to protect themselves and therefore need the Sertoli cells and peritubular cells within the seminiferous tubules, while the Leydig cells and immune cells in the interstitium play essential roles in rendering protection to the germ cells against viruses so as to regulate the immune responses to limit interfering with the germ cell development [77–79].

Testicular Inter-cellular Networks in Immune Privilege

Immune and Germ Cells Crosstalk

Testicular germ cells mainly interact with dendritic cells (DCs), testicular macrophages (t-Mφ), regulatory T cells (Tregs), effectors T cells (e-TCs), natural killer T cells (nk-TCs), and Mast Cells (MCs) [6, 13, 14, 46].

For DCs to have access to activated naïve T cells, they must undergo maturation process which consists of cytokines expression and lymphocyte homing receptors as well as increased expression of co-stimulatory molecules [80]. Homing receptors are able to direct DCs to the location of secondary lymphoid organs where T cells are developed [80]. Following their maturation, DCs influence the adaptive and innate T cell-dependent immunity, while the normal testicular DCs have been observed not to stimulate T cell proliferation contributing to the maintenance of testicular tolerogenic microenvironment to protect the allogenic germ cells [43]. In addition to DCs, testicular macrophages are vital players in germ cell protection and testicular immune modulation. As discussed earlier, there are two main subsets of macrophages in the testis. Another important testicular immune regulators are the Tregs which express Foxp3 within the testis as opposed to elsewhere in the body [81]. Furthermore, the Tregs play a vital role in peripheral tolerance by suppressing the activation and proliferation of e-TCs in the testes thereby restricting inflammatory responses that can potentially affect the germ cells. Yet another well-established testicular immunoprotectors are the nk-TCs, the major lymphocyte population that mediate germ cell apoptosis and phagocytosis of the cell debris [82]. Finally, the testicular MCs demonstrate a contrary role to its conventional functions in autoimmunity and inflammatory responses, playing as intermediary cells in testicular tolerance imparted by the Tregs [64].

The testicular somatic and immune cells also mediate germ cells apoptosis which needs intricate cellular programming to ensure a balance between viable and apoptotic milieu [16]. Germ cells initiate early programmed death in order to maximize viable germ cells for spermiogenesis and minimize unviable cells, as well as to bring the germ cells production in line with the Sertoli cell support for this process [83, 84]. Bax and Bcl-xL are two of the most potent indicators of gonadal apoptosis [85]. It has been shown that as the testis mature with onset of normal spermatogenesis during puberty, Bax concentration decreases and eventually is suppressed [86]. Moreover, expression of Fas and caspase 8 are necessary for mass apoptosis of germ cells. Temporary germ cell apoptosis during the onset of spermatogenesis in puberty is essential to regulate maturing germ cell number as per the capacity of Sertoli cells to support just a limited number of those [87]. These processes are supported by various resident immune cells-derived inflammatory mediators, such as cytokines and nitric oxide (NO) — specifically from testicular macrophages which are key factors regulating the proliferation of germ cells [88]. Low concentrations of NO have been shown to correlate with higher rate of germ cell apoptosis or halt of their proliferation [89]. This may explain why the interstitial space has a lower concentration of NO compared to the seminiferous tubules. Among the cytokines, IL-6, TNF-a, and soluble FasL also are inducers of germ cell apoptosis [90]. The soluble FasL may be produced from membrane-bound FasL by certain matrix metalloproteases from 'a disintegrin and metalloproteinase' (ADAM) family [91]. The pre-pubertal germ cells have been shown to express ADAM10 and 17 that perhaps aid their apoptosis at the time of first spermatogenic wave [42, 92]. Interestingly, evidences show that testicular T cells and Leydig cells may be an interstitial sources of the soluble FasL in the chronically inflamed testis, and these soluble FasL can enter the adluminal compartment of seminiferous tubule and thereby induce apoptosis of Fas-bearing germ cells [90].

Leydig Cell-Macrophage Crosstalk

The Leydig cells and testicular macrophages possess physical and functional crosstalk [41]. The Leydig cells are the most abundant testicular cell population in the interstitial space followed by the resident macrophages with ratio of 4:1 [93]. During the phase of post-natal development, macrophages have been shown to form inter-cytoplasmic digitations for close contacts with Leydig cells [94]. The digitations are made of cytoplasmic processes interleaved into macrophage intracellular channels, to aid inter-cellular transportation of various factors [95]. Testicular macrophages may stimulate Leydig cell steroidogenesis via secretion of certain factors, for example, 25-hydroxycholesterol that can be cleaved by Leydig cells specific steroidogenic enzymes, 17-B hydroxysteroid dehydrogenase representing a major steroidogenic step [96]. Thus, these macrophages are suggested to be essential for testicular testosterone production [48].

Besides the influence of macrophage over Leydig cells functions, it has been suggested that luteinizing hormone (LH), through its action on the Leydig cells, stimulates the macrophage proliferation during puberty and also maintains the macrophage population in the adult testis [6, 97]. Moreover, the Leydig cells derived androgens influence testicular macrophage population which in turn may modulate the activation of testicular lymphocytes [6, 56, 98]. It may be suggested that coordinated functions of the testicular cells along with the endocrine, exocrine, paracrine and immune components in the testis, help to maintain testicular immune homeostasis and render protection to the developing germ cells. However, this complex molecular crosstalk involved in testicular immune homeostasis is not completely understood and thereby need further in-depth research.

Sertoli Cell-Testicular Immune Cells Crosstalk

Sertoli cells aid functional maturation of testicular macrophages, receiving signals from the follicle-stimulating hormone (FSH) [99]. Sertoli cells also play a vital role in

modulating the functions of macrophages, NK cells as well as the dendritic cell to establish an immune suppressive environment [3, 100]. Sertoli cells produce transforming growth factors (TGF) β1 − β3 and indoleamine 2,3 dioxygenase (IDO), which are potent anti-inflammatory factors protecting the islet β-cell grafts [101, 102]. Sertoli cells also secrete granzyme B inhibitors. Since, the predominant pathway employed by the cytotoxic CD8 + T cells to kill the target cells is "granule exocytosis," that involves the release of perforin and granzyme B, the granzyme B inhibitors secreted by the Sertoli cells protect the allogenic germ cells from the immune-attack of the cytotoxic T cells [103]. They also secrete activin A and B, that help in initiating spermatogenesis as well as in inhibiting the expression of pro-inflammatory cytokines, (such as TNFs, IL-1, and IL-6) by the interstitial macrophages, NK cells, and dendritic cells [6, 104]. Moreover, Sertoli cells express programmed death receptor-1/programmed death ligand-1 (PD-1/PD-L1) which may inhibit T cell activation 105. Thus, multiple immunosuppressive mechanisms are mediated by the Sertoli cells via its interactions with various immune cells.

Role of Androgens in Testicular Immune Homeostasis

Reportedly, androgens have anti-inflammatory and immunosuppressive effects on the testes [106]. It has been reported that androgens have nongenomic actions on different cells including the immune cells and these actions are through membrane androgen receptors (G-protein coupled receptors) expressed on surfaces of the target cells (including the testicular immune cells). Activation of these receptors results in Ca^{2+} mobilization, secretion, and cytoskeleton modifications which in turn influence the secretory functions of testicular immune cells [107]. For long, studies have been showing the role of androgens in testicular immune privilege, such as the study by Head and Billingham from 1985 which reported that estrogen mediated inhibition of Leydig cell steroidogenesis in rats lead to rapid rejection of intratesticular allografts [108]. There are a batch of reports with similar findings [109–111]. Finally, it was later confirmed that androgens actually can also regulate the numbers of lymphocytes and macrophages in the testicular interstitium [56]. More recent reports suggest that androgens play a protective role in inflammatory conditions, such as orchitis, by suppressing the production of pro-inflammatory cytokines by testicular immune cells as well as restrict macrophage and T cells migration to the testis [9, 112]. Moreover, studies have shown that testosterone supplementation can induce expansion of protective CD4 + CD25 + regulatory T cells population [14, 113]. The transcription factor forkhead box P3 (Foxp3) is known to be a key regulator of regulatory T cell functions [114] and it is reported that the Foxp3 locus bears functional androgen receptor-binding site and androgen binding to this site may lead to epigenetic changes in the regulatory T cells [113]. Moreover, in an experimental autoimmune orchitis (that represent a type of testicular autoimmunity), it was shown that testosterone supplementation could significantly increase the number of CD4 + CD25 + Foxp3 + regulatory T cells in the testis [112], thereby confirming androgen-induced expansion of the protective regulatory T cell population to confer immune-privilege of the testis.

However, the limited number of such studies is not only not enough to firmly establish the mechanism of androgen actions in regulating testicular immune functions, but also the urgent need for more in-depth functional studies.

Conclusion and Future Perspectives

Regulation of testicular immune privilege is a complex mechanism. Herein, we discussed the current knowledge of the integrated functions of each testicular immune regulator in sequestering antigens to limit autoimmune responses, establishing a tolerogenic immune microenvironment in the testes by intricate cellular networking, actions of various immunomodulatory factors, thereby protecting the developing germ cells from immune attack. Disruptions of the immune privilege of the testes lead to autoimmune diseases, chronic orchitis, and subsequently to male sub- or infertility. Testes possess a special local innate immune system which is vital to initiate immune responses in case of pathogenic invasions in the testes. Suppression or downregulation of this testicular innate immunity helps to maintain the immune privilege. However, emerging evidence points towards the role of innate immune receptors, such as the pattern recognition receptors (PRRs) and their modulation in breaching of testicular immune privilege, which should be further investigated to reveal the appropriate etiologies and pathways involved in various immunogenic male infertility. The understanding of the testicular immune privilege, which is gradually being explored by more emerging evidence, will help in developing strategies to diagnose and manage immunogenic infertility.

Data Availability Not applicable.

Code Availability Not applicable.

Declarations

Ethics Approval Not applicable.

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