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REVIEW

Mechanisms of testicular immune privilege

Tao WANG, Xueying FENG, Daishu HAN (✉)

Department of Cell Biology, School of Basic Medicine, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100005, China

Abstract The testis exhibits a distinctive form of immune privilege to protect the germ cells from the host immune attack. The property of testicular immune privilege was originally attributed to the blood-testis barrier in the seminiferous epithelium, which sequesters antigens. Recent studies have uncovered several levels of immune control besides the blood-testis barrier involved in the privilege of the testis, including the mechanisms of immune tolerance, reduced immune activation, localized active immunosuppression and antigen-specific immunoregulation. The somatic cells of the testis, especially Sertoli cells, play a key role in regulating the testicular immune privileged status. The constitutive expression of anti-inflammatory factors in the testis by somatic cells is essential for local immunosuppression. Growing evidence shows that androgens orchestrate the inhibition of proinflammatory factors and shift cytokine balance toward a tolerogenic environment. Disruption of these protective mechanisms, which may be caused by trauma, infection and genetic factors, can lead to orchitis and infertility. This review article highlights the unique immune environment of the testis, particularly focuses on the regulation of testicular immune privilege.

Keywords immune privilege, testis, immunoregulation, spermatogenesis

Introduction

The concept of immunological privilege in mammals includes two aspects of notions: some tissues are predisposed to induce tolerance after being transplanted to an allogeneic recipient, and some tissues readily accept foreign cells without the induction of an immune rejection. The testis exhibits both the two aspects of immune privilege (Fijak and Meinhardt, 2006). At puberty, male germ cells enter meiosis and subsequently complex morphological formation of highly specialized spermatozoa. During this process, a large number of novel proteins are expressed. Therefore, the production of male gametes represents challenges to the immune system, as they are unique to the body and appear long after the establishment of immune competence. However, these unique antigens are tolerated by the testis. It is the testis itself that confers protection, as these autoantigens induce strong autoimmune responses when were injected elsewhere in the body (Tung et al., 1981). Initial consideration of the testis as an immune privileged site was substantiated experimentally when allografts placed into the interstitial space of the rat testis survived for indefinite time (Head et al., 1983). The transplantation of spermatogonial stem cells into germ-cell-depleted testis, even at interspecies, can restore spermatogenesis (Brinster, 2002). Similarly, ectopically transplanted allogenic testes under the kidney capsule or subdermal resist rejection without systemic immunosuppression in animals (Kuopio et al., 1989; Ma et al., 2004).

A general concept is that immune privilege is an evolutionary adaptation to protect vulnerable tissues or cells from loss of function (Filippini et al., 2001). For the testis, this protection means safeguarding male reproductive ability. Immune privilege could not attribute to absent lymphatic drainage. In fact, the majority of immune privileged tissues possess effective lymphatics. Therefore, immune privilege is assumed to be related to the blockade of immune surveillance due to the presence of barriers that restrict immune cell access, such as the blood-testis and blood-brain barriers. This mechanism cannot be fully responsible for the privileged status, since active immune feature cells exist behind these tissue barriers, for example, the microglial cells in brain (Wekerle et al., 1987), macrophages in testicular interstitium and Sertoli cells within seminiferous tubules. Moreover,

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Correspondence: Daishu HAN
E-mail: dshan@ibms.pumc.edu.cn

immune responses to antigens occur in immune privileged organs (Hedger, 2002). Growing evidence shows that immune privilege is controlled by multiple mechanisms, including active local immunosuppression and adaptive (antigen-specific) immunoregulation within the privileged tissues. In testis, the antigen-specific immune responses prevent reactions against auto-antigens associated with spermatogenesis. This protection certainly extends to prevent allograft rejection within the testis environment. Following will discuss the mechanisms of this protection and its significance for the normal testicular function.

The structural and functional basis of testicular immune privilege

There are multiple levels of immunological control in the testis. The blood-testis barrier is originally thought as the mechanism of testicular immune privilege. This traditional view has several serious short-comings. For examples, immune cells circulate through the interstitial tissue where grafts are placed (Head et al., 1983), and the blood-testis barrier cannot completely impede immune cells or antibodies, which are able to enter the seminiferous tubules via the downstream reproductive tracts and rete testis (Dym and Romrell, 1975). Therefore, testicular structure is not an essential determinant of immune privilege. In fact, immune privilege in the testis is conditional. Allografts and xenografts enjoy prolonged survival in the testes of some species, including rat, mouse and guinea-pig (Head and Billingham, 1985). However, similar studies using ram and monkey have not been successful (Setchell et al., 1995). There is evidence that some species, such as the rabbit and mink, are more susceptible to testicular autoimmune reactions than other species (Pelletier et al., 2009), and that rat and mouse strains also show variable susceptibility to experimental autoimmune orchitis (Teuscher et al., 1987). These observations suggest that genetic differences play a role in the integrity of immune privilege.

Structure of the testis

The testis has two major functions: the generation of sperms (spermatogenesis) and the production of sex steroid hormones (steroidogenesis). The two functions are performed in distinct regions. Spermatogenesis takes place within seminiferous tubules, and steroidogenesis is fulfilled by Leydig cells in the interstitial compartment between the tubules (Fig. 1).

The seminiferous tubules are highly coiled, which originate and terminate at the rete testis. Each tubule is surrounded by myoid peritubular cells, which together with Sertoli cells secrete the components of the basement membrane enclosing the seminiferous epithelium. The columnar Sertoli cells extend from the basal lamina toward the lumen of the tubules. They are responsible for the physical support of the germ cells, providing essential nutrients and growth factors.

The most components of the testicular interstitial compartment are Leydig cells that produce steroid hormones (prominently testosterones). The interstitium also contains blood vessels and immune cells such as macrophages, dendritic cells, lymphocytes (Fig. 1). The various extracellular matrix proteins are important in cell-cell interactions within the testis (Dym, 1994), particularly growth factor binding proteins modulates the availability of growth factors to the target cells (Schlatt et al., 1997). Exit of the testis is the rete testis, a transition zone between the testis and the epididymitis. However, the blood-testis barrier is terminated in this area. Therefore, spermatozoa are no longer protected from the autoimmune attack in this zone, thus certain forms of autoimmune orchitis are primarily observed in the rete testis (Itoh et al., 2005).

Blood-testis barrier

The blood-testis barrier is formed by tight junctions between neighboring Sertoli cells localized in the seminiferous epithelium, which restricts the passage of proteins through the intercellular spaces. This structure divides the seminiferous tubule into two distinct compartments: the basal compartment carring spermatogonial stem cells and early stage of primary spermatocytes, and the adluminal compartment possessing meiotic pachytene and secondary spermatocytes as well as haploid spermatids. The blood-testis barrier creates a unique environment for the progress of meiosis and morphogenesis of germ cells, and to protect the developing germ cells from the immune attack. During meiosis and morphogenesis, germ cells express a large number of novel antigens that could initiate systemic immune responses. Given that germ cell autoantigens are also present in the basal compartment in spermatogonia and early spermatocytes (Saari et al., 1996), it is accepted that the blood-testis barrier alone cannot be fully responsible for the testicular immune privileged status. Moreover, allografts that were placed in the interstitial space, a location outside the blood-testis barrier, showed extended survival without immune rejection (Head and Billingham, 1985). Therefore, some other mechanisms, besides physical separation, must exist to maintain the testicular immune privilege.

The function of testicular immune privilege

Immune privilege in the testis is conditional. Allografts and xenografts can survive in the testes of some species, such as rat, mouse (Head and Billingham, 1985). This survival does not dependent on the reduced temperature of the testis (Ma et al., 2004; Selawry and Whittington, 1984). Moreover, the testes do not always exhibit privilege properties as donor tissues. Mouse testis allografts under the kidney capsule enjoy prolonged survival (Bellgrau et al., 1995), but adult rat testes to kidney allografts do not (Statter et al., 1988). These differences might suggest that some factors in testicular

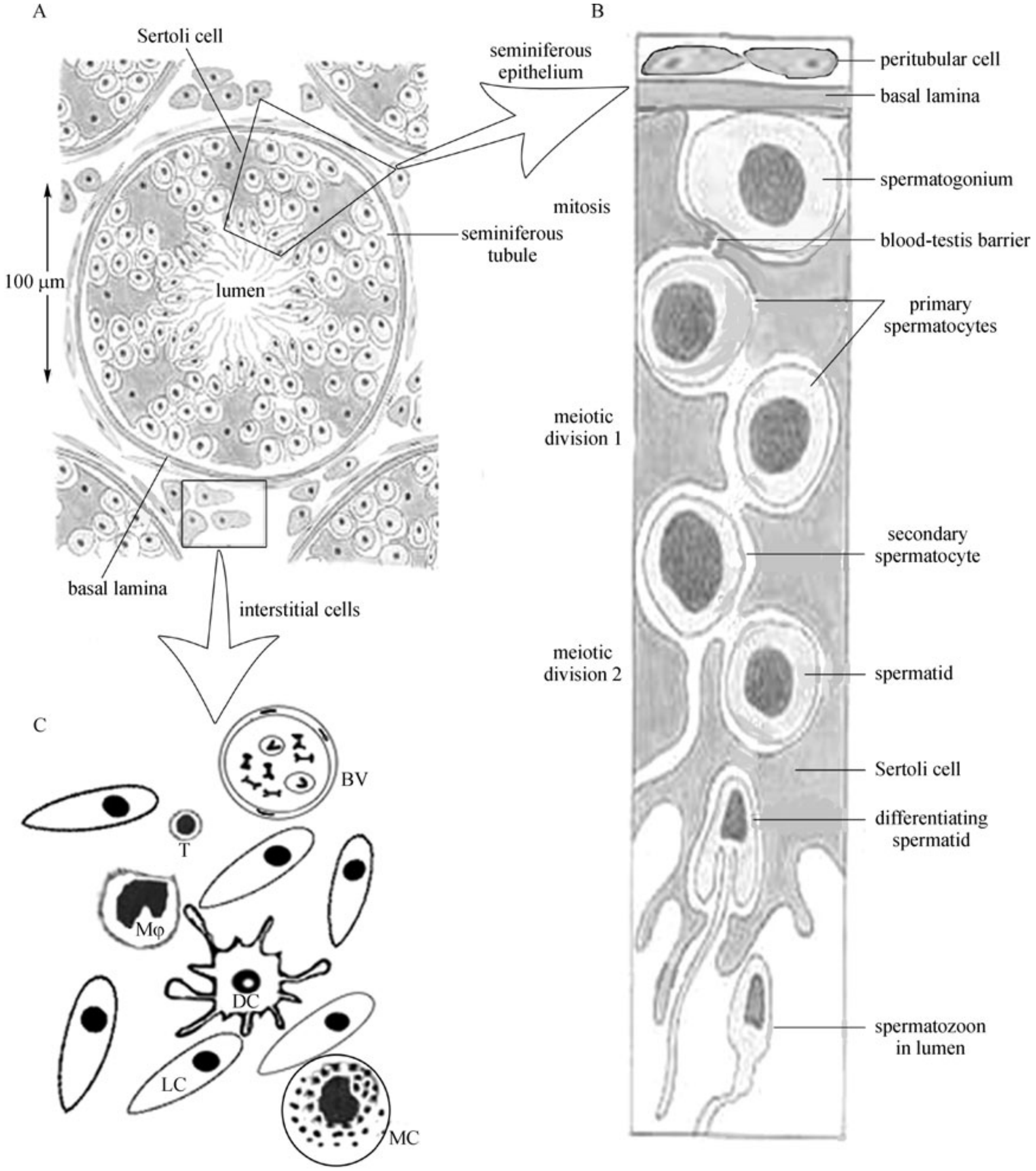

Figure 1 Schamatic drawing of micrographs of a mammalian testis. (A) The seminiferous tubules within the developing germ cells are in intimate association with Sertoli cells, and the interstitial compartment between the tubules. The Sertoli cells extend from the basal lamina to the lumen of the seminiferous tubule, and are required for the development of the germ cells by providing physical support and nutrients. Myoid peritubular cells surround the seminiferous tubules and maintain the integrity of the tubules by secreting the components of the basal lamina enclosing the tubules. (B) Amplification of the seminiferous epithelium shows different stages of germ cells. (C) Amplification of the interstial compartment shows that the interstitium contains mainly Leydig cells (LC), which produce androgens, and various types of immune cells including macrophages (Mφ), dendritic cells (DC), T cells (T) and mast cells (MC), as well as blood vessels (BV) (Alberts et al., 2001).

architecture, the capacity to accommodate surgical trauma and special challenges facing transplantation, are variable among species.

An intact testis structure is not necessary for immune privilege. Testicular Sertoli cells display inherent immunosuppressive properties, which can support the survival of cells from other tissues, such as pancreatic islet cells, when were co-transplanted (Suarez-Pinzon et al., 2000; Selawry and Cameron, 1993). On the other hand, allogeneic transplantation of mouse germ cells into the seminiferous tubule of recipients induces intratubular immune responses (Kanatsu-Shinohara et al., 2003). However, germ cell transplantation has been successfully performed between pigs, goats and dogs (Dobrinski, 2005; Kim et al., 2008). These observations provide further evidence for inter-species differences in testicular immune privilege.

Overall, previous studies supported that genetic elements play a role in the maintenance of testicular immune privilege, and that different species may lead to different outcomes. Even so, some commonality of mechanisms for controlling the immune privileged status of the testis might be exited in different species. One should keep this in mind when supporting data will be obtained. Notably, immune privilege of the testis does not mean that immune response is absent in this tissue, but rather is reduced.

Cellular mechanisms of testicular immune privilege

Testicular immune privilege should involve several levels of immune control, including antigen sequestration by the blood-testis barrier, local immunosuppression, and the role of immune cells that are resided in interstitial space of the testis. However, as we understand further the systemic immune mechanisms that are involved in the maintenance of testicular immune privilege, another question arises: whether the testis-specific cells play roles in driving this protection? In this regard, more attentions have been paid to Sertoli, Leydig, peritubular, and even germ cells, which appear to be also responsible for the testicular immune privilege.

Role of interstitial immune cells in regulating testicular immune privilege

Macrophages

All kinds of immune cells can be found in the interstitial compartment (Wang et al., 1994). Among them, macrophages represent a major population of the interstitial immune cells. In rats and mice, macrophages represent about 20%–25% the interstitial cells (Hutson, 1994; Hedger, 2002). Under pathological conditions or in the regressive testis of seasonal breeders, such as swan, macrophages can enter the seminiferous tubules for phagocytic clearance of apoptotic germ cells (Breucker, 1978; Rival et al., 2008).

Although mechanisms underlying the role of testicular macrophages in the immune privilege of the testis are not clear, there is no doubt that they play a role in the maintenance of the testicular immune privileged status. Testicular macrophages display a reduced capacity to produce inflammatory factors compared with those from other tissues (Kern et al., 1995), and exhibit immunosuppressive properties (Kern and Maddocks, 1995). Even so, the testis is able to acquire an inflammatory response to allo- and auto-antigens. This response can be contributed by the testicular somatic cells, such as Sertoli, Leydig and peritubular cells, via producing proinflammatory modulators (Meinhardt et al., 1996; Meinhardt et al., 2000; Zeyse et al., 2000).

Different macrophage populations have been demonstrated in the testis (Hutson, 1994). At least two subsets of macrophages can be discerned: one that can be recognized by an antibody ED (murine homolog of human CD63), and the other that can be positively stained by the antibody ED2 (human CD163). The ED2$^+$ macrophages represent a resident subset, which is the majority (~ 80%) of testicular macrophages. The ED1$^+$ macrophages presumably belong to the circulating subset of monocytes/macrophages, which represents a minor (~ 20%) proportion of testicular macrophages. The ED2$^+$ resident macrophages do not initiate inflammatory responses, which may be involved in the maintenance of immune privilege. Whereas, the ED1$^+$ monocyte/macrophages initiate prominently inflammatory process and migrate into the testis during acute and chronic inflammation to overcome the immune privilege (Gerdprasert et al., 2002a; Rival et al., 2008).

Macrophages express the major histocompatibility (MHC) antigens, which are critical to activate antigen-specific T cell responses, and are effective antigen-presenting cells (APCs) (Haas et al., 1988). They, combined with dendritic cells (DCs) that are typical the most potent APCs are responsible for the activation of antigen-specific T cell responses, and must contribute to the unique immune environment of the testis.

Although the local factors that control the balance of ED2$^+$ resident macrophages and ED1$^+$ circulating monocytes/macrophages is still unknown, growing evidence supports the role of the testicular somatic cells, including Leydig, Sertoli and pertubular cells, via producing cytokines (Hedger and Meinhardt, 2003; Huleihel and Lunenfeld, 2004). It can be speculated that the testicular somatic cells and immune cells together provide an environment that protects the germ cells from autoimmune attack. In this concert, TGF-β and activin A may play an important role, by inhibiting specific immune responses to testicular self-antigens, in maintaining immune privilege (O'Bryan et al., 2005).

Dendritic cells

Dendritic cells are the most important APCs. They play a major role in initiating immune responses of lymphocytes, the effect cells of the adaptive immunity. DCs not only activate lymphocytes, but also inhibit autoimmune responses by tolerizing T cells to antigens (Banchereau and Steinman, 1998). DCs have been recently demonstrated with a minor number (~ 1×10^5) in normal rat testis (Sanchez et al., 2006). However, in experimental autoimmune orchitis (EAO) models, the number of DCs in the testis was significantly increased. The general quantity of testicular DCs is much lower than macrophages in similar circumstances (Meinhardt et al., 1998). In spite of a potential important role of DCs in maintaining the balance of the testicular immune status, the mechanisms remain elusive. Various heat shock proteins (Hsps, e.g. Hsp60 and Hsp70) were recently characterized as testicular auto-antigens, which may represent a mechanism for the role of the testicular DCs in the activation of autoreactive lymphocytes (Fijak et al., 2005). Hsp70, which is released by necrotic cells resulting from infection or injury, triggers autoimmunity (Millar et al., 2003). Therefore, it is reasonable to hypothesize that the immature DCs, normally participates in maintaining immune privilege, become mature status by sensing self-antigens like Hsp70. The mature DCs may overcome immune privilege by local activation and expansion of autoreactive T cells.

Lymphocytes

There is evidence that antigen-specific control mechanisms are involved in testicular immune privilege. The interstitium of the testis also contains T lymphocytes (Hedger, 1997). Injection of soluble antigen into the testis induces specific suppression of T cell-mediated responses against the antigens (Li et al., 1997).The deletion or inactivation of antigen-specific T cells represents a mechanism of active immunosuppression, and is a feature of testicular immune privilege. In normal immune response, T cell activity is controlled by activation-induced cell death mediated by Fas/Fas ligand (FasL) interaction (Ju et al., 1995). FasL is expressed by Sertoli cells, and may be responsible for the deletion of activated T cells, thereby suppressing locally adaptive immunity (Fig. 2). However, this hypothesis remains somewhat controversial, since the presence or absence of FasL on epithelial cells in some human tissues and animal models does not correlate well with immune privileged status (Xerri et al., 1997).

The studies on pancreatic islet cell allografts in the mouse testis showed that the activated T cells are targeted for destruction when they enter the testis environment, whereas graft antigen-specific regulatory T cells (Tregs) are produced (Dai et al., 2005; Nasr et al., 2005). $CD4^{+}CD25^{+}$ Tregs are a subset of T cells that exhibit profound immunosuppressive function. Particulars, Tregs and nature killer (NK) cells are found within the testicular interstitium under physiologic conditions (Jacobo et al., 2009). These intratesticular lymphocytes should contribute to the testicular immune privilege.

Mast cells

Recent studies showed that mast cells regulate innate and adaptive immunity, particularly the development of autoimmune diseases (Benoist and Mathis, 2002). Upon activation, mast cells produce numerous potent proinflammatory mediators that may influence onset of the diseases. Through release of proinflammatory factors (e.g. TNF-α), mast cells can affect vascular permeability, thus the blood-brain barrier opening, which in turn provides inflammatory cell traffic (Bebo et al., 1996). Mast cells are distributed in the interstitial spaces of the testis, and may regulate steroidogenesis by Leydig cells (Aguilar et al., 1995). There is evidence that increased mast cell number in the testis is associated with man infertility (Hussein et al., 2005). A little has been known about the role of mast cells in the testicular immune privilege. The low number of mast cells in the normal testis may be one of mechanisms underlying the limited inflammation.

Involvement of testis-specific cells in the testicular immune privilege

Leydig cells

Leydig cells represent a major cell population in the interstitial compartment of the testis. Leydig cells are critical endocrine cells that produce androgens for both the seminiferous tubule compartment to regulate spermatogenesis and the peripheral circulation to extra-testicular androgen target organs (Diemer et al., 2003). Several studies have showed that rat Leydig cells exhibit high antiviral activities in response to viral infections (Dejucq et al., 1998; Melaine et al., 2003), whereas human Leydig cells display relatively weak the antiviral abilities (Le Tortorec et al., 2008).The mechanisms of the Leydig cell-initiated immune responses against microbial pathogens remain elusive.

Growing evidence indicates that there is a physical and developmental relationship between Leydig cells and macrophages (Hedger, 2002), and Leydig cells regulate the expansion of the testicular macrophages (Raburn et al., 1993). Moreover, the lymphocytes in the testis are affected by the endocrine environment (Raburn et al., 1993; Hedger and Meinhardt, 2003). It has been known that androgens have immunosuppressive roles, which contribute the immunological differences between the sexes (Cutolo et al., 2004). A role of androgens in regulating immune privilege is first suggested by the observation that the blockade of Leydig cell androgen production rapidly rejected intratesticular allografts (Head and Billingham, 1985). The role of androgens in suppressing graft rejection in the testis is equivocal (Cameron et al., 1990). The precise role of androgens in directing the testicular immune responses has not been firmly established. Notably, intratesticular testosterone concentration is 10-fold higher than serum (Jarow et al., 2005), which is far greater than that needed for the maintenance of normal spermatogenesis (Sharpe et al., 1988). The high local testosterone concentration is likely involved in the maintenance of the testicular immune privilege. It seems that testosterones play immunosuppressive function by regulating the balance of pro- and anti-inflammatory cytokine expression in Sertoli, Leydig, and peritubular cells and do not affect directly testicular leukocytes, as androgen receptor has not been found in the testicular immune cells.

Peritubular cells

Myoid peritubular cells surround the seminiferous tubules and provide structural support to the integrity of the tubules. They contain contractile elements that help to transport the immotile spermatozoa into the epididymis. The peritubular cells do not form a tight barrier to prevent diffusion, but secrete the components of the basal lamina enclosing the contents of the seminiferous epithelium. Moreover, these myoid cells secrete a large number of cytokines, which presumably regulate the functions of immune cells.

Sertoli cells

Sertoli cells are only somatic cells within the seminiferous tubules. They acquire with a columnar sharp extending from the basal lamina toward the lumen of the tubules (Fig. 1). Sertoli cells constitute the main structural element of the seminiferous epithelium, and are responsible for the physical support of germ cells in addition to providing essential nutrients and growth factors.

Sertoli cells were recognized to have immunosuppressive activities nearly two decades ago (Wyatt et al., 1988; De Cesaris et al., 1992). As already noted, Sertoli cells are able to provide an immunoprotective environment for some allografts and xenografts in co-transplantation experiments (Suarez-Pinzon et al., 2000; Sanberg et al., 1996). This protection could not be due to a physical barrier formed by Sertoli cells in the testis, but seems to result from inherent properties of the cells. The factors secreted by Sertoli cells, and the molecules expressed on their surface are most likely participated in the immunoprotection of Sertoli cells. Previous studies have demonstrated that graft survival is associated with the production of FasL and anti-inflammatory cytokine transforming growth factor β_1 (TGFβ_1) by Sertoli cells (Bellgrau et al., 1995; Suarez-Pinzon et al., 2000; Fallarino et al., 2009). Moreover, Sertoli cells express complement regulatory proteins, which participate in the regulation of their immunoprotective function (Head et al., 1983). Other important immunoregulatory molecules expressed by Sertoli cells include inhibitors of granzyme B and ligand B7-H1, which are involved in the destruction of cytotoxic lymphocytes (Sipione et al., 2006) and apoptosis of antigen-specific T cells (Dal Secco et al., 2008) respectively. Notably, an recent study demonstrated that TAM (Tyro3 Axl and Mer) receptor tyrosin kinase subfamily are inhibitors of the innate immune responses (Rothlin et al., 2007; Lemke and Rothlin, 2008).We found that TAM receptors and their ligand Gas6 are expressed in mouse Sertoli cells and suppress Toll-like receptor-mediated testicular innate immune responses (Wang et al., 2005; Sun et al., 2010). The precise mechanisms that Sertoli cells protect grafts and create immune privileged status of the testis still remain to be clarified.

Molecular mechanisms of testicular immune privilege

Toll-like receptor (TLR)-initiated testicular innate immune responses

An immune privileged status does not mean that there is no effective immune response in the site. In fact, the testis is no more susceptible to allo- and auto-antigens than other tissues and remainder of the genital tracts (Krieger, 1984). Innate immunity plays a critical role in the defense against infections in the testis in consequence of that the specific adaptive immune response must be suppressed for the immune privilege.

TLRs are pattern recognition receptors that recognize pathogen-associated molecular patterns (PAMPs). The activation of TLRs by PAMPs represents one of the most important mechanisms of immune responses against pathogens (Takeda and Akira, 2005). Upon the recognition of PAMPs, TLR signaling induces potent innate immune responses through adaptor molecules myeloid differentiation factor 88 (MyD88)-dependent pathway and Toll/IL-1 receptor (TIR) domain containing adaptor protein inducing IFN-β (TRIF)-dependent(or MyD88-independent) pathway to activate transcription factors, nuclear factor (NF)- κB, activator protein 1 (AP-1), and interferon regulatory factors (IRFs). TLR-mediated innate immune response induces proinflammatory factors including chemokines and cell adhesion molecules to recruit macrophages, neutrophils and nature killer (NK) cells, particularly secretion of specific antimicrobial products, such as the interferons and defensins (Selsted and Ouellette, 2005).

TLRs are expressed predominantly on antigen-presenting cells (APCs) such as DC and macrophages, which is opposed to the heterologous and polymorphic antigen-recognition receptors that mediate T and B lymphocyte responses. Recent studies have demonstrated that testicular macrophages and Sertoli cells express TLRs and initiate testicular innate responses (Bhushan et al., 2008; Riccioli et al., 2006; Wu et al., 2008). In these cells, TLR-initiated inflammatory responses exhibit unique features that seem to be consistent with the immune privilege (Bhushan et al., 2008; Winnall et al., 2009). In addition to the testicular macrophages and Sertoli cells, we have found Leydig cells and some stages of germ cells also express various TLRs and initiate innate immune responses (Tao Shang et al. unpublished data). Moreover, defensins, which are small (3–4 kDa) peptides that disrupt membrane of microbes, are produced in the testis (Com et al., 2003). The expression of abundant TLRs and defensins in testicular cells are agreement with an important role for innate immunity in this tissue.

Local immunosuppression mechanisms

Active immunosuppression, such as deletion or inactivation of antigen-specific T cells, involves in the maintenance of testicular immune privilege. In physiologic condition, T cell activity is controlled by a process of activation-induced cell death through the Fas/FasL interaction (Ju et al., 1995). FasL is expressed on epithelial cells in the immune privileged tissues, including testicular Sertoli cells, and induce apoptosis of activated T cells, thus suppress locally adaptive immune responses (Fig. 2). However, this hypothesis remains controversial, since the expression of FasL on target tissue does not control induction of experimental autoimmune uveitis (Wahlsten et al., 2000).

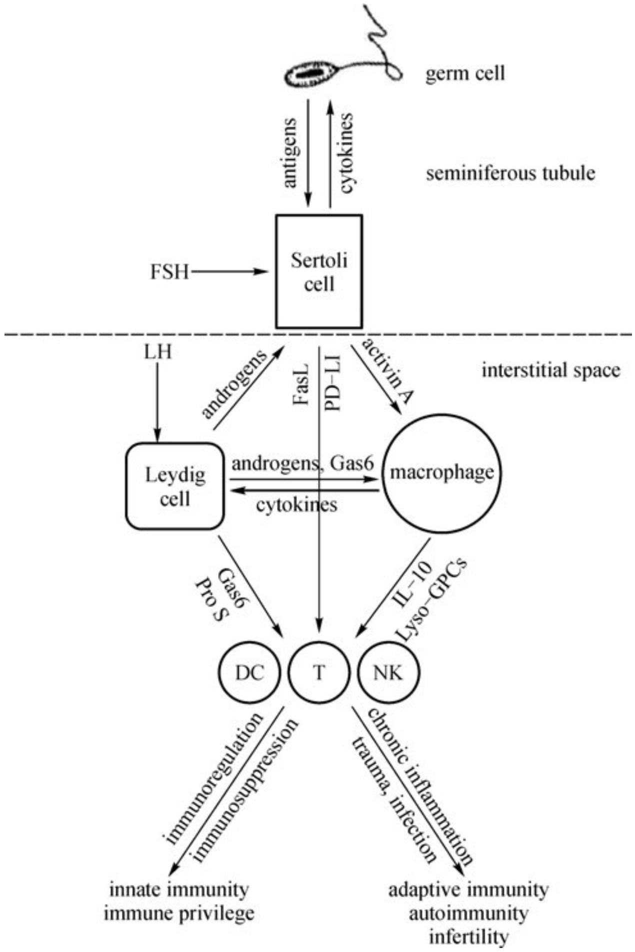

Figure 2 A model of intercellular communication in the testis leading to immune privilege. Sertoli cells play a central role, under the influence of follicle-stimulating hormone (FSH) from pituitary, androgens from Leydig cells and germ cell antigens. Testicular macrophage function is regulated by Leydig and Sertoli cells via paracrine molecules. The infiltration and activity of the other immune cells including T lymphocytes (T), dendritic cells (DC) and mast cells (MC) were regulated by cytokine from Leydig, Sertoli cells and mcrophages.

Indoleamine 2, 3-dioxygenase (IDO) prevents T cell-driven complement activation and inflammation in allogeneically unmatched pregnancies (Mellor et al., 2001), suggesting that IDO may be involved in immune privilege. Furthermore, IDO produced by trophoblasts inhibits T cell-mediated autoimmunity, and stimulates Treg functions (Mellor and Munn, 2004). A recent study has shown that IDO participates in the protection of islet allografts by Sertoli cells (Fallarino et al., 2009).

A recent study demonstrated that lyso-glycerophosphatidylcholines (lyso-GPCs) presented in interstitial fluid inhibit T cell activity, thus contribute to testicular immunosuppression (Foulds et al., 2008). Although certain lyso-GPCs regulate the function and survival of T cells, the mechanisms remain to be clarified. Moreover, it is worthwhile to further study the cell-specific expression and regulation of lyso-GPCs in the testis.

Programmed death receptor-1 (PD-1) is an immunoreceptor molecule-based inhibitory motif-containing transmembrane protein, and programmed death ligand-1(PD-L1, also named B7-H1) is functional ligand of PD-1. PD-1 is expressed in T cells, and its activation by PD-L1 mediates T cell tolerance (Keir et al., 2006). An recent study demonstrated that PD-L1 is constitutively expressed in the testis and contributes to the long-term survival of islet allografts transplanted in the testis, suggesting that PD-1/PD-L1 system may represents a mechanism of testicular immune privilege (Cheng et al., 2009).

TAM receptors are the latest identified subfamily of receptor tyrosine kinases, which include three members: Tyro3, Axl and Mer (TAM) (Hafizi and Dahlbäck, 2006a). Two close relative vitamin K-dependent proteins, the product of growth arrest-specific gene 6 (Gas6) and protein S (a blood anticoagulant cofactor) are biologic ligands of TAM receptors (Hafizi and Dahlbäck, 2006b). TAM receptor triple knockout mice are male infertility due to progressive loss of germ cells (Lu et al., 1999; Chen et al., 2009). Gene targeting mutation studies showed that TAM receptors are essential regulators of homeostasis of the immune system through inhibiting TLR-initiated innate immune responses (Lu and Lemke, 2001; Rothlin et al., 2007).We previously demonstrated that TAM receptors are abundantly expressed in testicular Sertoli cells, whereas Gas6 is prominently expressed in Leydig cells (Wang et al., 2005). Recently, we provided evidence that TAM signaling suppresses Sertoli cell-initiated testicular innate immune responses (Sun et al., 2010). Based on these findings, we speculate that Gas6/TAM system may be critical in regulating the immune privileged status of the testis, and we are now investigating this possibility directly.

Endocrine and paracrine regulation of testicular immune privilege

The effects of hormones on the testicular immunity

In addition to a close physical contact between Leydig cells and macrophages, the development of these two cell populations is regulated by each other (Hedger, 2002). Luteinizing hormone (LH) may control macrophage expansion in the puberty testis and the maintenance of macrophages in the adult testis through acting on Leydig cells (Raburn et al., 1993). There is also evidence that follicle-stimulating hormone (FSH) regulates the maturation of testicular macrophages via Sertoli cells (Duckett et al., 1997).

Furthermore, other type of immune cells in the testis can be affected by the testicular endocrine environment (Hedger and Meinhardt, 2000). Notably, there is data suggest that the immune cells in the testis dependent on the function of a mature testis, rather than an effect of the hormones on the immune cells (Meinhardt and Hedger, 2010).

The production of androgens (mainly testosterones) is a major function of Leydig cells. Androgens exhibit immunosuppressive activities, which contribute to the different of immune responses between the sexes (Cutolo et al., 2004). A role of androgens in the testicular immune privilege was arisen by the observation that inhibition of Leydig cell androgen production rejected rapidly intratesticular allografts (Head and Billingham, 1985). This function of androgens was controversial, as the later studies demonstrated that prolonged intratesticular islet allograft survival is not dependent on local androgen production (Selawry and Whittington, 1988; Cameron et al., 1990). However, recent data suggest a protective role of androgens in experimental autoimmune orchitis in rats. Therefore, the role of androgens in regulating the testicular immune status remains to be clarified.

Paracrine cytokine regulation of the testicular immune environment

Several immunoregulatory cytokines are expressed in the testis, particularly those with immunosuppressive activities, such as transforming growth factor β (TGF-β) family and activin A (Avallet et al., 1994; Buzzard et al., 2004; O'Bryan et al., 2005). TGF-βs contribute to the testicular immune privilege through their immunosuppressive activities (Pöllänen et al., 1993). TGF-β1 facilitates Sertoli cells to support graft survival in co-transplantation experiments (Suarez-Pinzon et al., 2000). On the other hand, TGF-β_3 regulates the breakdown of blood-testis barrier (Lui et al., 2003). Therefore, the role of TGF-β family members in regulating the testicular immune privilege is likely complex. Activins are structural homology to the TGF-βs and exibit stimulatory and inhibitory effects on germ and Sertoli cell proliferation. Activin A is principally produced by Sertoli cells and peritubular cells and can be stimulated by IL-1 (Okuma et al., 2005).

IL-1 is an important mediator of inflammation in the testis. A recent study showed that IL-1α facilitates blood-testis barrier opening by affecting the actin cytoskeleton (Sarkar et al., 2008). Unlike IL-1α, IL-1β seems to be feebly produced by the testis under physiologic conditions. However, an upregulation of IL-1β was observed in the testis inflammatory conditions, which might contribute to the injury of the testis (Guazzone et al., 2009).

IL-6 promotes potently inflammatory events via the expansion and activation of T cells. IL-6 is produced by most testicular cells, including somatic cells and germ cells in normal rats (Potashnik et al., 2005). In LPS-induced acute testicular inflammation model, IL-6 expression is upregulated. In experimental autoimmune orchitis (EAO), IL-6 expression in testicular macrophages is significantly increased (Rival et al., 2006). Notably, circulating monocytes ($ED1^{+}$) that recently arrived to the testis express IL-6 at a high level in comparison to resident testicular macrophages ($ED2^{+}$). This suggest a distinct role of the two types of macrophages in mediating inflammatory responses. *In vitro* study shows that the presence of exogenous IL-6 induces germ cell apoptosis (Theas et al., 2003).

Tumor necrosis factor α (TNF-α) is the most potent proinflammatory cytokine. Within the normal seminiferous tubules, TNF-α is prominently systhesized by germ cells (De et al., 1993), and regulates Sertoli cell-tight junction dynamics in the rat testis (Siu et al., 2003). Various types of the interstitial cells such as macrophages, mast cells synthesize TNF-α (Xiong and Hales, 1993). TNF-α regulates testicular function oppositely in health and disease conditions. It protects germ cells from apoptosis at a physiologic low concentration in the normal testis. In contrast, TNF-α behaves as an apoptotic factor to induce germ cell death under inflammatory conditions (Theas et al., 2008).

Chemokines are a large family of small cytokines with chemoattractive activities, which are grouped into the two major subfamilies: CC ligand (CCL) and CXC ligand (CXCL). Of the CCL subfamily, CCL2 (MCP-1) is present in the testis at physiologic low level (Gerdprasert et al., 2002b). CCL2 is expressed by Leydig and peritubular cells, and can be regulated by IL-1, TNF-α, and IFN-β. In response to TLR activation, CCL2 was upregulated in Sertoli cells (Riccioli et al., 2006). Injection of LPS *in vivo* induces an elevated CCL2 level in the interstitial fluid in rats (Gerdprasert et al., 2002b). Accordingly, an increased CCL2 was observed in the testicular fluid of EAO model (Guazzone et al., 2003). Of CXCL subfamily, CXCL10 is expressed in rat Leydig cells, and upregulated by IL-1α, TNF-α, and IFN-γ (Hu et al., 1998). In addition, CXCL1 and CXCL10 were induced by Sendai virus in rat testicular macrophages, Sertoli, Leydig and peritubular cells (Aubry et al., 2000). In contrast, these chemokines were not detected in rat germ cells. Leukocyte infiltrations are involved in the inflammatory reaction in the inflamed site, in which the chemokines play a contral role.

Another anti-flammatory cytokine, IL-10 reduces inflammation, autoimmunity and spermatogenic damage in mouse model of EAO (Watanabe et al., 2005). During inflammation, IL-10 is likely produced by intratesticular immune cells, rather than other testicular cells. The production of immunosuppressive cytokines supports the speculation that the testicular immune cells contribute to immune privileged status of the testis.

Conclusions and perspectives

The testis is a unique organ according to its major functions of spermatogenesis, steroidogenesis and a special immune environment, which are affected one another through a network of interactions. Many unique gene products are synthesized by germ cells during spermatogenesis. Various mechanisms exist in the testis to protect germ cells from autoimmune attack by antigen-specific immune responses, namely “immune privilege”. The mechanisms underlying testicular immune privilege are not well understood. The data presented here highlight the achievements of the studies on the testicular immunity and point out the issues that are worthwhile to further investigate. Although the testis is considered as a typical immune privileged site, chronic orchitis-retated male infertility is frequently observed. In fact, human with about 15% of diagnosed male infertility is related to immunity, while the contribution of impaired immune environment to idiopathic infertility (30% of all cases) remains unknown (McLachlan, 2002).

How failure of immune privilege balance in the testis cause male infertility is a more speculative issue. The challenges for further study on the testicular immunity are to determine the contribution of local mechanisms to the maintenance and disruption of the immune privileged status. Understanding of these fundamental processes of the testis, together with manipulation of pro- and anti-inflammatory molecules, may provide appropriate therapeutic approach to control testicular inflammation and relevant male infertility.

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