# Immune Privilege of the Testis: Meaning, Mechanisms, and Manifestations

**Mark Peter Hedger**

**Abstract** The mammalian testis belongs among a small number of tissues that can unambiguously be called "immunologically privileged," as demonstrated by the ability to tolerate not only testicular autoantigens but also allo- and xenoantigens experimentally located within the testis environment. The mechanisms underlying this privilege remain poorly understood compared with more intensively studied models of immune privilege, such as the eye and feto-uterine unit, but evidently share key functional elements with these tissues. While physical structures like the blood-testis barrier have been implicated, antigen sequestration, aberrant lymphatics, or impeded immune cell access is not the underlying cause of testicular immune privilege, and it is increasingly evident that privilege involves active immunoregulation and local immunosuppression. More specifically, the unique somatic cells of the testis, the Sertoli cells of the seminiferous epithelium, and the steroidogenic Leydig cells, together with the large resident testicular macrophage population, have been directly implicated in suppressing or regulating immune responses to antigens located within the testicular environment. It is increasingly evident that these immunological control mechanisms also impinge upon, and may even participate in the regulation of, normal testicular function, spermatogenesis, and steroidogenesis. Conversely, failure of immune privilege is a significant cause of disease in the male tract, leading to chronic inflammation, infertility, and pain.

**Keywords** Autoimmune orchitis • Blood-testis barrier • Hypogonadism • Infertility • Leydig cell • Sertoli cell • Sperm antibodies • Spermatogenesis • Steroidogenesis • Testicular macrophages

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## 1 Introduction

Maturation of the immune system occurs during fetal and early neonatal life. Arguably the most important aspect of this maturation is acquisition of the ability of the immune system to recognize or, more accurately, to ignore self-antigens. This ignorance involves the deletion or inactivation of antigen-specific T cell and B cell clones that are capable of interacting with antigens that are normally expressed by the host, leading to immunological tolerance of these self-antigens (Nossal 1994; Mueller 2010). Spermatogenesis, which is the production of mature sperm from stem cell precursors in the testis, occurs exclusively within the postpubertal period. Consequently, this is one of the few biological processes whereby large numbers of novel autoantigens appear after the maturation of the immune system, paralleled only by the fetal allograft during pregnancy and the emergence of new antigens that may be associated with tumors and viral infections. Protection of the developing spermatogenic cells from the immune system throughout adult life is a physiological imperative, and the testis has established mechanisms to facilitate this protection. The following review provides a brief outline of the current evidence for testicular immune privilege, the mechanisms underlying this privilege in so far as they are currently understood, and the consequences for testicular function, which may impact upon male fertility and health.

## 2 Critical Aspects of Testicular Structure and Function

The mammalian testis comprises two functional tissue compartments: the seminiferous tubules, where the spermatogenic stem cells (spermatogonia) develop into mature sperm (spermatozoa), and the interstitial tissue, which contains the steroidogenic Leydig cells, as well as the testicular vasculature and its innervation (Fig. 1). The testicular lymphatics also entirely invest the interstitial tissue, generally comprising discrete lymphatic vessels but forming open lymphatic sinusoids that are contiguous with the interstitial fluid space in some species, most notably the principal laboratory rodents (rats and mice)(Fawcett et al. 1973; Setchell et al. 1990). The seminiferous tubules possess a complex epithelium, comprised of the developing spermatogenic cells and supporting epithelial cells, called Sertoli cells, bounded by a basement membrane and a layer of specialized myoid-like peritubular cells. The tubules are entirely avascular but are closely surrounded by an elaborate and extensive network of arterioles, venules, and capillaries (Suzuki and Nagano 1986; Ergün et al. 1994). Within the seminiferous epithelium, the spermatogonia rest on the basement membrane at the base of the Sertoli cells, slowly undergoing regular mitotic divisions. At precisely defined intervals, the spermatogonia undergo an asymmetric mitotic division, with one daughter cell becoming committed to the process of spermatogenesis. After several further mitotic divisions, the committed spermatogonia enter into meiosis, thereby becoming spermatocytes, and are displaced toward the lumen of the tubule between the adjacent Sertoli cells. Extensive structural reorganization and differentiation of the spermatocytes occurs,

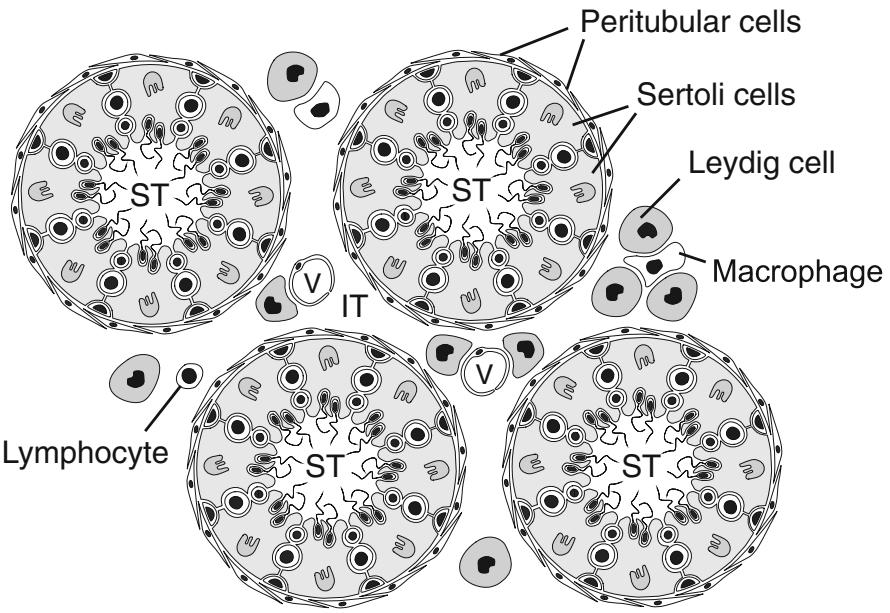


**Fig. 1** Diagrammatic cross section of the mature testis, showing the major cellular and structural features relevant to testicular immunoregulation. The testis comprises two major compartments, the seminiferous tubules (ST) and the interstitial tissue (IT). The seminiferous epithelium comprises the epithelial Sertoli cells, with the developing spermatogenic cells lying between these cells and progressing toward the lumen of the tubule as they mature. The tubules are surrounded by a basement membrane and a layer of peritubular cells. Within the interstitial tissue are found numerous Leydig cells, macrophages, and lymphocytes, as well as the testicular blood vessels (V). The testicular lymphatics are also located within the interstitial tissue. In most species, the testicular lymphatics are discrete vessels lined with a continuous endothelium, but in the rat and mouse, they form open sinusoids that are continuous with the interstitial tissue space

producing, after two meiotic divisions, haploid spermatids. The spermatids undergo further elaboration of their cytoplasmic and nuclear contents, including the appearance of several entirely new structures unique to the sperm, such as the acrosome and flagellum, and exchange of most of the nuclear histones for a new set of nucleoproteins, called protamines. As the spermatogenic cells mature through this process, they progress toward the lumen of the tubule, eventually becoming embedded in the apical cytoplasm of the Sertoli cells and are held in place by unique, highly specialized intercellular junctional complexes (Kerr et al. 2006). Within the epithelium, several generations of differentiating spermatogenic cells form multiple cell layers of increasing complexity and development moving toward the tubular lumen. Finally, the Sertoli cells release the spermatids into the lumen, at the same time stripping away and digesting most of the excess cytoplasm of the cell, producing free spermatozoa, which bear little physical resemblance to the spermatogonial stem cells from which they arose. More importantly, in the context of this chapter, the molecular and biochemical composition of these cells is also quite distinct from that of the spermatogonia or any somatic cells of the testis (Chalmel et al. 2007; Aitken and Baker 2008).

Each seminiferous tubule is connected at both ends to a collecting structure within the testicular parenchyma, called the rete testis, which is directly connected to the genitourinary tract via a series of excurrent ducts. These ducts carry the sperm into the adjacent epididymis, where the sperm mature, undergoing further differentiation and modifications that render them capable of movement and fertilization, and are stored prior to ejaculation. At the time of ejaculation, sperm are rapidly propelled from the epididymides via the vasa deferentia into the urethra to join with the secretions of the accessory sex organs, most notably those of the seminal vesicles and prostate. Consequently, the testis and the epididymis are the tissues where large numbers of spermatogenic cells or sperm are present for long periods of time, although sperm also may be found within the lumen of the vas deferens, urethra, and the accessory organs, even outside the time of ejaculation (McClinton et al. 1990; Peirce et al. 2003).

Crucially, spermatogenesis, with the appearance of spermatocytes, spermatids, and spermatozoa in the testis, first occurs at the time of puberty. Puberty is driven by an increase in secretion from the anterior pituitary of the gonadotropins luteinizing hormone (LH), which drives the maturation of the Leydig cells and stimulates testosterone production, and follicle-stimulating hormone (FSH), which stimulates maturation of the Sertoli cell (Plant and Wichel 2006). Both FSH and testosterone regulate the activity of the Sertoli cell, which is essential for the onset of spermatogonial commitment to meiosis and maintenance of spermatogenesis in the adult testis. Prior to puberty, for a period of about 12 years in the human male, the only spermatogenic cells that are present in the testis are the spermatogonial stem cells. Consequently, numerous antigens that are uniquely associated with the developing and mature sperm, as well as many molecules produced by the Sertoli cells during active spermatogenesis, will have escaped the normal mechanisms of tolerance induction, particularly through clonal deletion of lymphocytes, during fetal and early neonatal life and may be seen as foreign by the host immune system. Moreover, at the time of puberty, the Leydig cells undergo extensive transformation from a relatively quiescent precursor into a highly active steroid-producing cell (Nistal et al. 1986). Several enzymes, cellular structures, and other molecules that are unique to mature steroid-secreting cells increase dramatically within the interstitial tissue at this time.

## 3 Origins and Significance of Testicular and Sperm Autoimmunity

Presumably as a result of this dramatic transformation of the testis at the onset of sexual maturity, many testicular autoantigens appear to be incompletely protected from the immune system. The spermatogenic cells, in particular, are highly immunogenic, as indicated by a relatively high incidence of "spontaneous" fertility-suppressing sperm antibodies in the male tract, affecting approximately one in every 200 men in the developed world (Baker et al. 1983; Lenzi et al. 1997).

In most such cases, leakage of sperm from the male reproductive tract due to congenital malformations, physical trauma, infection-related inflammation, or surgical interventions, such as vasectomy, causes an immunological response, which leads to the formation of sperm antibodies (Linnet 1983; Tung 1987). Genetic predisposition toward autoimmunity also appears to be a significant factor (Baker et al. 1985; Paschke et al. 1994). These antibodies target the sperm for destruction in the male and female reproductive tracts or interfere with their ability to swim toward, bind , and fertilize the egg. Although these antibodies may resolve themselves with time, many affected men experience permanent immunological infertility, which may require assisted reproduction to bypass the antibody-mediated impediments (Clarke et al. 1997). In some animal models, and possibly some human patients, antibody reactions may be followed by autoimmune orchitis and damage to the seminiferous epithelium, potentially resulting in complete sterility (Tung and Alexander 1980; Salomon et al. 1982; Kohno et al. 1983; Roper et al. 1998; Schuppe et al. 2008). Such susceptibility is genetically determined and is most commonly associated with the autoimmune polyglandular syndromes, which are due to genetic disruption of critical tolerance-inducing mechanisms, leading to damage of multiple endocrine organs, targeting steroidogenic cells in particular (Maclaren et al. 2001). Certain infections and inflammation in the male tract, most notably mumps orchitis, are also a potential cause of destructive testicular autoimmunity (Krieger 1984; Philip et al. 2006). The incidence of autoimmune orchitis and resulting infertility in humans appears to be rare, although it is almost certainly considerably underestimated. It is likely, for example, that idiopathic, noninfectious or sterile scrotal, pelvic, or perineal pain, a condition that affects many men particularly in older age, may involve underlying autoimmunity in the reproductive tract, including testicular autoimmunity (Pannek and Haupt 1997; Rivero et al. 2007).

Given the susceptibility of testicular and sperm autoantigens to immunological attack, one may be tempted to ask why responses to these antigens are so relatively infrequent. The answer lies in the fact that the male reproductive tract provides a unique immunological environment for sperm. It is generally accepted that the male reproductive tract constitutes an element of the mucosal immune system and that typical mechanisms responsible for tolerance in other mucosal tissues are involved (Clifton et al. 1992; Czerkinsky et al. 1999; Knee et al. 2005). However, as outlined in the remainder of this chapter, there is considerable evidence that the testis also possesses a number of unique immunological features that single it out for particular interest and have led to its classification as a tissue of specific immunological privilege.

## 4 The Meaning and Limits of Testicular Immune Privilege

The term “immune privilege” tends to have different meanings for different researchers. The most widely encountered definition is that of a site where lymphatic drainage or immune cell access is restricted, and sequestration of antigens of

the central nervous system behind the blood-brain barrier is usually considered to be the best example of this phenomenon (Carson et al. 2006). However, the original definition of immune privilege more specifically refers to a site where normal rejection responses against foreign tissue grafts, specifically allografts or xenografts, are reduced or prevented (Barker and Billingham 1977; Head and Billingham 1985b). These are functional definitions, promulgated prior to the emergence of modern principles of immunoregulation and tolerance. A more contemporary definition of immune privilege, which would encompass both of these traditional concepts as well as more recent immunobiology, can be stated as "the extended survival of cells expressing antigens that under normal circumstances should provoke an immune response, as well as the mechanisms that contribute to this survival" (Hedger 2007).

Several lines of evidence indicate that the testis is immune privileged by this definition. As outlined in the previous section, the argument from logic is that the developing spermatogenic cells need to be protected from the host immune system. The most direct evidence for immune privilege in the male reproductive tract, however, comes from a number of studies that have shown extended allograft and xenograft survival within the testis. Admittedly, this increased graft survival has only been convincingly demonstrated in laboratory rodents, including rats, mice, and guinea pigs (Ferguson and Scothorne 1977; Bobzien et al. 1983; Head et al. 1983a). Attempts to replicate this in other species, such as sheep and monkeys (Maddocks and Setchell 1988; Setchell et al. 1995), have been less successful, and even in rodents, it is clear that the parameters that underlie successful graft survival into the testis are poorly understood. Various factors, including tissue complexity; vascularization and differences in lymphatic organization; the size, health, and type of graft; the underlying immunogenetics of the donor and host; and even the surgical procedures employed, no doubt influence intratesticular graft success rates. Nonetheless, regardless of the reasons why graft survival has occurred in some studies but not in others, the testis certainly fits the classical criteria of immune privilege as proposed by Billingham and colleagues, as a site where foreign grafts may enjoy extended survival relative to other sites (Barker and Billingham 1977; Head and Billingham 1985b).

There is evidence that testicular tissue and, more specifically, some testicular cells have inherently immunologically privileged characteristics that may make them more amenable to transplantation (Neaves and Billingham 1979; Statter et al. 1988; Barten and Newling 1996). Mouse testis allografts have been reported to survive under the kidney capsule (Bellgrau et al. 1995), and allogeneic transplantation of spermatogenic cells, which were subsequently able to undergo spermatogenic development, have been performed in immunologically intact pigs, goats, bulls, and dogs (Dobrinski 2005; Herrid et al. 2006; Kim et al. 2008). As is the case with grafts into the testis, however, such grafts of testicular tissue have not been universally successful for reasons that are still not understood. More significant is the observation that isolated testicular cell preparations containing Sertoli cells can be successfully transplanted into various tissues across both allogeneic and xenogeneic barriers (Mital et al. 2010). The mechanisms and implications of this unique property of the Sertoli cell will be discussed in more detail later.

## 5 Mechanisms Underlying Immune Privilege in the Testis

In spite of the absence of most spermatogenic cells at the time of the establishment of central tolerance, mechanisms of tolerance are clearly important for preventing testicular autoimmunity. This is indicated by the association of hypogonadism with the autoimmune polyglandular syndromes, which are caused by genetic defects in $CD4^+CD25^+$ Treg cell development and in the autoimmune regulator (Aire) gene that regulates thymic expression of various tissue-specific autoantigens, particularly autoantigens of the endocrine system (Ramsey et al. 2002; Kriegel et al. 2004). However, this hypogonadism is primarily due to autoimmunity against the steroidogenic (Leydig) cells within the testicular interstitium, and the damage to spermatogenesis and sperm production may be a secondary effect (Maclaren et al. 2001). In the Aire-deficient mouse, testis function appears to be normal, but sperm antibodies develop, leading to loss of fertility, and the epididymis contains numerous inflammatory infiltrates (Hubert et al. 2009). In several strains of rats and mice, thymectomy at 3 days of age, which causes a reduction in regulatory T cell subsets in the adult, results in spontaneous epididymo-vasitis and, eventually, orchitis (Lipscomb et al. 1979; Taguchi and Nishizuka 1981; Tung et al. 1987a). Studies in mice, rats, and rabbits have shown that tolerance to testicular antigens, leading to orchitis, epididymitis, and vasitis, can also be broken by active immunization with testicular or sperm homogenates or, more significantly, by passive transfer of T cells from actively immunized animals (Tung et al. 1971; Tung and Fritz 1984; Tung et al. 1987b; Mahi-Brown and Tung 1989). In some strains of mice, rats, and rabbits, autoimmune epididymo-orchitis may also develop following vasectomy (Alexander and Tung 1977; Taguchi and Nishizuka 1981; Flickinger et al. 1990), and there is some evidence that this may also occur in a small subset of human vasectomies (Goldacre et al. 2007). Overall, the evidence suggests that active tolerance is involved in protecting critical testis autoantigens and, apparently, in protecting sperm autoantigens in the rest of the reproductive tract (epididymis and vas deferens), thereby contributing to immune privilege in the testis.

On the other hand, studies on graft rejection responses in the rat and mouse testis suggest that immune privilege may also involve a failure to recognize antigens or to subsequently activate immunity when these antigens are first encountered within the testis environment. It has been shown that even long-standing intratesticular allografts are rapidly rejected when the host is sensitized to the graft antigens external to the testis, either by active immunization or by grafts of donor tissue to the skin, for example (Head et al. 1983a; Head and Billingham 1985a). This is consistent with the fact that both active immunization and passive transfer of activated lymphocytes from immunized animals to naïve recipients can cause testicular antibody formation and autoimmune orchitis (Tung et al. 1987b; Mahi-Brown and Tung 1989).

Experimental evidence that the testis is able to specifically regulate immunity comes from studies showing that introduction of antigens via the testicular route is able to induce suppression of T cell-mediated immunity against the injected

antigens. For example, prior injection of the relevant dominant antigens into the testis is able to ameliorate the experimental induction of autoimmune uveoretinitis, adjuvant-induced arthritis, or autoimmune encephalomyelitis (Li et al. 1997; Ditzian-Kadanoff 1999; Veräjänkorva et al. 2002). The mechanisms underlying this acquired immune deviation (AID) have not been studied in any detail in the testis, but they are probably analogous to mechanisms that operate in other well-characterized immunologically privileged sites, such as the eye and brain. Immune privilege in these tissues have been shown to involve the localized production of immunosuppressive cytokines, such as transforming growth factor-β (TGF-β) and interleukin-10 (IL-10), and induction of antigen-specific immunoregulatory lymphocytes, specifically Treg cells, natural killer (NK) T cells, and γδT cells (Wilbanks et al. 1992; Sonoda and Stein-Streilein 2002; Ashour and Niederkorn 2006). Studies on pancreatic islet allografts in the mouse testis have shown that activated and memory T cells directed against graft antigens are selectively deleted within the testicular environment and that graft antigen-specific Treg cells are preferentially induced (Dai et al. 2005; Nasr et al. 2005). Moreover, NK cells, NKT cells, and Treg cells are strongly represented among the lymphocytes found within the interstitial tissue of the rat and mouse testis even under normal conditions (Tompkins et al. 1998; Jacobo et al. 2009) (Hedger, unpublished data). These data suggest that antigen-specific immunity within the testicular environment tends to favor an immunoregulatory or tolerogenic response, rather than a cell-mediated immune response that would normally be associated with graft rejection and autoimmunity.

Another characteristic feature of the testis relevant to immunity is the large population of resident macrophages within the interstitial tissue (Fig. 1) (Hedger 2002). The macrophages tend to be closely associated with the Leydig cells, with which they maintain intimate structural and functional interactions (Miller et al. 1983; Raburn et al. 1993; Wang et al. 1994; Gaytan et al. 1996). Much smaller numbers of dendritic cells are also present (Itoh et al. 1995; Fijak et al. 2005; Rival et al. 2006). The testicular macrophages and dendritic cells express major histocompatibility complex (MHC) class II antigens, indicating their capacity to interact with and activate $CD4^+$ T cells, although there is evidence that this expression may be impaired to some extent (el-Demiry et al. 1987; Tung et al. 1987b; Wang et al. 1994). In studies from the rat and the mouse, the majority of macrophages have been shown to be alternatively activated, with greatly reduced capacity for lymphocyte activation and proinflammatory cytokine production, and enhanced production of immunoregulatory cytokines, most notably IL-10 (Kern et al. 1995; Bryniarski et al. 2004; Winnall et al. 2011). Given that macrophages are the pivotal cells in the initiation of inflammation and subsequent immune responses, the functional properties of the testicular macrophages are entirely consistent with the manifestations of testicular AID. The functional activity of the dendritic cells of the testis remains to be examined in detail, but existing data suggest that they are immature and, therefore, will tend to be tolerogenic (Rival et al. 2007).

In summary of this section, all the existing data point to the conclusion that immune responses within the testicular environment tend toward suppression of

antigen-specific cell-mediated responses, favoring tolerogenic immune responses instead. On balance, this indicates that testicular immune privilege is actively maintained and regulated.

## 6 The Testicular Environment and Immune Privilege in the Testis

There are two widely held misconceptions about immune privilege as it pertains to the testis. The first misconception is that the testis has deficient or abnormal lymphatics and that restrictions on the movement of immune cells and other effectors contribute to an absence of immune responses within the testis. In fact, it has been clearly established in several species, including the experimental rodents used to study immune privilege, that the testis possesses effective lymphatics draining directly to local lymph nodes (Fawcett et al. 1973; Moller 1980; Head et al. 1983b; Itoh et al. 1998). Macrophages, dendritic cells, and lymphocytes, and even eosinophils and mast cells (Anton et al. 1998), are found throughout the testicular interstitium, which encompasses the intratesticular lymphatic vessels and is the site where surviving intratesticular grafts are normally located. Moreover, both MHC class I and class II antigens are expressed throughout the interstitial tissue (el-Demiry et al. 1987; Haas et al. 1988; Pöllänen et al. 1992; Wang et al. 1994), and antigens introduced into the testis interstitium can induce antigen-specific T cell responses (Head et al. 1983b; Ditzian-Kadanoff 1999; Nasr et al. 2005). As a corollary, it should be noted that the significantly lower temperature of the testis in most mammalian species does not appear to contribute to immune privilege (Selawry and Whittington 1984; Head and Billingham 1985a).

The second misconception is the role of the blood-testis barrier. In contrast to the blood-brain barrier, this barrier is not located at the level of the vascular endothelium of the testis. In the testis, the vascular endothelium is comparatively permeable to circulating immune cells, as already noted, as well as immune effector molecules, such as circulating cytokines, immunoglobulin, and complement (Yule et al. 1988; Hedger and Hettiarachchi 1994; Pöllänen et al. 1995; McLay et al. 1997). The blood-testis barrier is actually created by highly specialized tight junctions between the adjacent Sertoli cells, which serve to effectively separate the seminiferous epithelium into an adluminal and a basal compartment (Fig. 2) (Setchell et al. 1969; Dym and Fawcett 1970; Meng et al. 2005). The adluminal compartment of the seminiferous epithelium, where the majority of the developing spermatogenic cells and their unique autoantigens are located, constitutes a highly specialized biochemical environment that is also characterized by reduced MHC expression and the absence of immune cells, immunoglobulin, and other immune effector molecules (el-Demiry et al. 1987; Haas et al. 1988; Pöllänen et al. 1992). This contributes to reduced immunological activity against spermatogenic cells in the adluminal compartment, and disruption of the blood-testis barrier is a critical

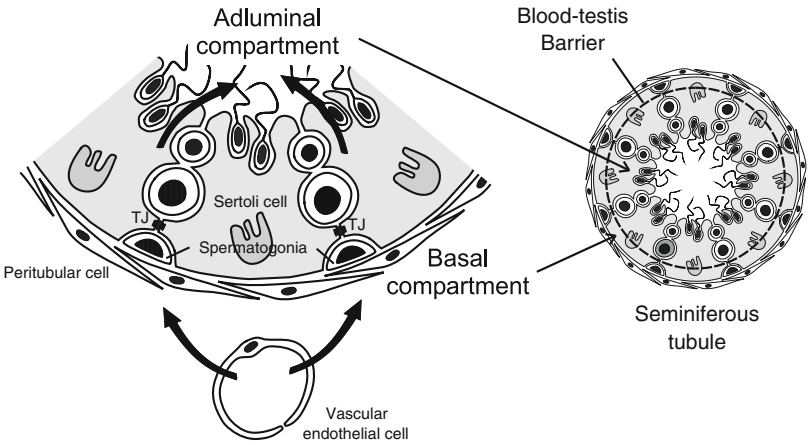


**Fig. 2** The location and structural features of the blood-testis barrier. The blood-testis barrier is created by highly specialized occluding tight junctions (TJ) located between adjacent Sertoli cells in the basal region of the seminiferous epithelium. These junctions entirely obstruct the space between the Sertoli cells and effectively separate the tubule environment into a basal compartment (containing the spermatogonia and some early spermatocytes), the composition of which is largely determined by the circulation (*arrows*) and interstitial tissue products, and the adluminal compartment (containing the spermatocytes, spermatids, and spermatozoa), the composition of which is determined almost exclusively by secretions of the Sertoli cells (*arrows*). Immune cells and immune effector macromolecules, including complement, antibody, and cytokines, are normally unable to traverse this barrier

step in the onset of autoimmune orchitis and infertility (Kohno et al. 1983; Yule and Tung 1993; Itoh et al. 2005; Meng et al. 2011). Nonetheless, immunogenic sperm autoantigens are also found on spermatogenic cells located in the basal compartment of the epithelium without provoking immunity (Yule et al. 1988), and recurrent disruption of the blood-testis barrier, as occurs in seasonally reproducing animals, does not typically cause overt autoimmunity (Pelletier 1986). Moreover, the functional blood-testis barrier is limited to the epithelium of the seminiferous tubules—the epithelium of the straight tubules linking the seminiferous tubules to the rete testis and the rete testis itself lacks the specialized tight junctions found between adjacent Sertoli cells and appears to exhibit only the typical tight junctions associated with mucosal epithelia (Osman and Plöen 1978; Ghabriel et al. 2002). Accordingly, these epithelia tend to be more permeable to immunoglobulin and to lymphocytes (Koskimies et al. 1971; Dym and Romrell 1975; Knee et al. 2005; Naito and Itoh 2008) and represent the initial sites of orchitis in the activated lymphocyte transfer model of autoimmune orchitis in mice (Tung et al. 1987b; Yule and Tung 1993).

The preceding observations regarding the testicular lymphatics and blood-testis barrier further highlight the fact that immune privilege is an active process maintained by the testis, rather than a simple property of structural elements that

might restrict the movement of immune cells and other effectors within the testis. Furthermore, it is the somatic cells of the testis that have been most directly implicated in regulating testicular immune privilege.

Regulation of the testicular macrophage population has been extensively studied in both rats and mice. These studies have shown that recruitment and maintenance of the resident testicular macrophage population is controlled by the Leydig cells, although this does not appear to involve testosterone (Raburn et al. 1993; Duckett et al. 1997b; Meinhardt et al. 1998). The recruitment is gonadotropin regulated, as removal of LH by various means also causes macrophage numbers to decline, suggesting that this is a function of the mature Leydig cell (Gaytan et al. 1994; Duckett et al. 1997a; Duckett et al. 1997b). In fact, testicular macrophage numbers increase rapidly during puberty, in parallel with the increase in mature Leydig cell numbers at this time (Hardy et al. 1989; Ariyaratne and Mendis-Handagama 2000). The actual mechanisms involved are still unclear, but in rats and mice, these two cell types are physically connected, forming clusters with highly specialized interdigitations between them (Miller et al. 1983; Hutson 1992). On the other hand, evidence suggests that the function of the testicular macrophages is regulated by the Sertoli cells, involving FSH-dependent mechanisms (Duckett et al. 1997a). Thus existing data suggest that recruitment of macrophages and their unique phenotype are functions of the mature testis under indirect gonadotropin regulation, through both the Leydig cells and the Sertoli cells (Fig. 3). In turn, the number of lymphocytes appearing within the testicular interstitial tissue is related to the size and activity of the macrophage population (Wang et al. 1994; Hedger et al. 1998; Hedger and Meinhardt 2000). Surprisingly, the presence of developing spermatogenic cells, which are also dependent upon the activity of the Leydig and Sertoli cells, does not appear to be essential either for maintenance of the testicular macrophage population (Meinhardt et al. 1998) or for persistence of testicular immune privilege defined by intratesticular graft survival (Selawry and Whittington 1984; Head and Billingham 1985a; Whitmore et al. 1985).

While the effects that the Leydig cell exerts on immune cell activity in the testis appear to be largely indirect, the Sertoli cell appears to play a much more immediate role in controlling intratesticular immunity. Not only do these cells have inherent immunosuppressive activity in lymphocyte cultures (Wyatt et al. 1988; Selawry et al. 1991; De Cesaris et al. 1992), they also show unique favor as transplants and are even able to provide protection for allogeneic and xenogeneic grafts of other cell type transplanted along with them (Selawry and Cameron 1993; Sanberg et al. 1996; Suarez-Pinzon et al. 2000). This protection does not depend upon the formation of tight junctions or barriers and appears to be due to properties inherent to the Sertoli cell itself (Mital et al. 2010). A number of immunoregulatory proteins produced by these cells have been identified: inhibitors of complement and granzyme B activity (O'Bryan et al. 1990; Sipione et al. 2006; Lee et al. 2007); lymphocyte-inhibiting molecules, such as Fas ligand (CD95L)(Bellgrau et al. 1995; Sanberg et al. 1997), indoleamine 2,3-dioxygenase (Fallarino et al. 2009), nonclassical MHC antigens (Slukvin et al. 1999; Ryan et al. 2002), and the inhibitory coreceptor B7-H1 (Dal Secco et al. 2008); and immunoregulatory cytokines, most

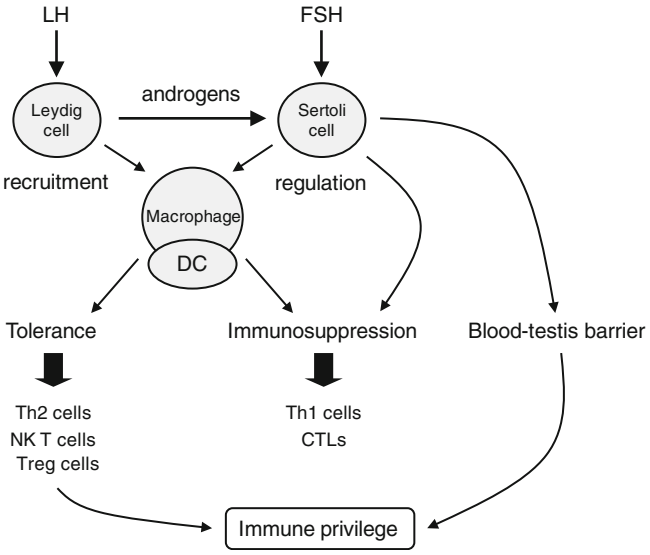


**Fig. 3** Summary of the putative mechanisms controlling immune responses within the testis. The immunological environment of the testis is regulated by the gonadotropins secreted from the anterior pituitary: luteinizing hormone (LH), which acts on the Leydig cells, and follicle-stimulating hormone (FSH), which acts on the Sertoli cells. In response to stimulation by LH, the Leydig cells produce androgens, particularly testosterone, which are necessary to support mature Sertoli cell functions. These functions include the maintenance of the tight junctions that comprise the main structural elements of the blood-testis barrier. The Leydig cells are responsible for recruiting macrophages into the interstitial tissue. Under the influence of the testicular environment, chiefly mediated by the Sertoli cells, the resident macrophages adopt an alternatively activated phenotype, characterized by reduced proinflammatory activity and preferential production of immunoregulatory cytokines, such as IL-10. Dendritic cells (DC) within the testicular interstitium appear to be functionally immature. The Sertoli cells, resident macrophages, and dendritic cells regulate the development of lymphocytes circulating through the testis, promoting the activity of regulatory T cells (e.g., Th2 cells, NKT cells, and Treg cells) and suppressing the activity of Th1 cells and cytolytic T cells (CTLs). This results in active tolerance to antigens encountered within the testis (and its draining lymph nodes) and inactivation of antigen-specific effector cells directed against testicular antigens, culminating in immune privilege

notably TGF-β and activin A (Skinner and Moses 1989; Suarez-Pinzon et al. 2000; Okuma et al. 2005). The relative importance of each of these Sertoli cell products toward maintaining testicular immune privilege remains to be established, but identifying the critical mechanisms that confer the ability to locally suppress graft rejection responses on the Sertoli cell may have considerable impact upon transplantation medicine.

Lipidic molecules with immunoregulatory or immunosuppressive activity, including testosterone, several of the eicosanoids, and some lysophosphatidylcholines, are produced by various testicular cell types. Testosterone, as well as other androgens, produced by the Leydig cells have immunosuppressive effects on macrophage and lymphocyte activity that are believed to contribute to

gender-discordant immune functions, such as the reduced susceptibility to certain autoimmune diseases in men and differences in innate and adaptive immunity between the sexes (Grossman 1985; Wichmann et al. 1997; Rettew et al. 2008). Testosterone concentrations within the testis are more than an order of magnitude higher than in the circulation or in other tissues and are also elevated in the epididymis (Jean-Faucher et al. 1985; Turner et al. 1985). Although early studies were equivocal concerning the relationship between intratesticular androgens and graft survival in the testis (Whitmore and Gittes 1978; Head and Billingham 1985a; Selawry and Whittington 1988; Cameron et al. 1990), there is evidence that androgens inhibit the progression of autoimmune orchitis in experimental models of the disease (Fijak et al. 2011). Furthermore, specific deletion of the androgen receptor on the Sertoli cell leads to disruption of the blood-testis barrier and an increase in intratesticular leukocytes and antibodies against spermatogenic cell antigens (Meng et al. 2011). Conversely, estrogens appear to promote intratesticular inflammation and have an inhibitory effect on graft survival in the testis (Head and Billingham 1985a; Li et al. 2006). Eicosanoid biosynthesis, which occurs in most testicular cell types, leads to production of several immunoregulatory and anti-inflammatory molecules, particularly prostaglandins E and D (Sorrentino et al. 1998; Winnall et al. 2007), although their importance in regulating immune responses in the testis has yet to be established. The presence of several medium chain-length lysophosphatidylcholines, which are produced during eicosanoid biosynthesis and several other processes of phospholipid metabolism, in the fluid of the testicular interstitial tissue has been shown to be responsible for the profoundly suppressive effect of these fluids on lymphocyte activity in vitro (Foulds et al. 2008).

Altogether, it appears that immune privilege in the testis, and possibly in the rest of the male reproductive tract, is maintained by a unique testicular environment that controls immune cell activity, inducing and maintaining peripheral tolerance and suppressing adaptive immunity in a tissue-localized manner (Fig. 3). This regulation involves specific hormone-dependent actions of the unique somatic cells of the testis, the Sertoli and Leydig cells, rather than simple sequestration of antigens or restriction of immune cell access.

## 7 The Consequences of Testicular Immune Privilege

The existence of immune privilege has important implications for both normal testicular function and male reproductive disease (Fig. 4). Although the testis is a common site for relapsing leukemia following therapy, which may be attributable to its unique immunological environment (Hudson et al. 1985; Kim et al. 1986), the testis does not display an increased susceptibility to tumors or infections compared with other tissues. In fact, infections of the testis are relatively rare in comparison with more distal tissues of the male reproductive tract (Krieger 1984). The intensity of inflammatory responses in the testis may be reduced, due to the unique regulatory

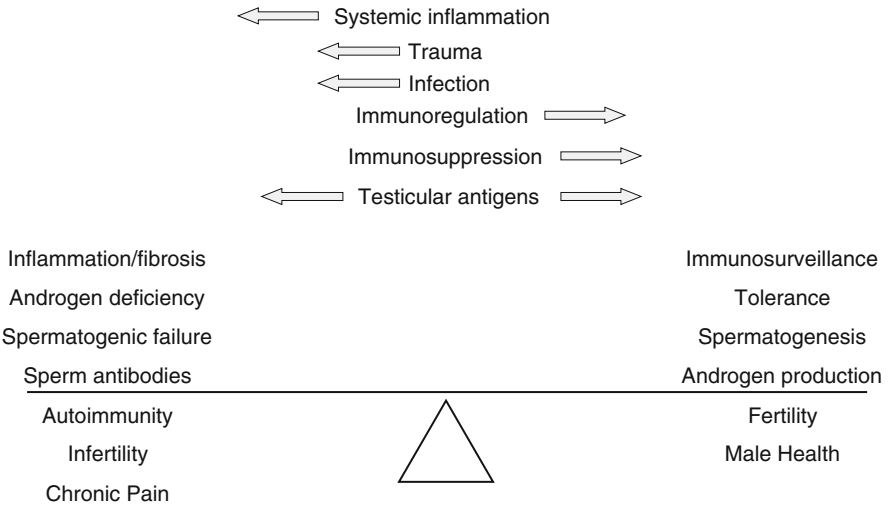


**Fig. 4** The balance between disease and normal function in the testis. Testicular function is dependent upon a precise balancing act involving the immune system, maintained by testis-specific immunoregulatory processes. The manifestation of this control is immune privilege with respect to testicular autoantigens, which may be extended to foreign antigens as well, leading to extended graft survival. The balance may be tilted toward reproductive disease by genetic predisposition, together with precipitating events, such as systemic inflammation, reproductive tract trauma, or infection

properties of the testicular macrophages, and antigen-specific immunity may be compromised, but it appears that the ability of the testis to resist and clear infections is intact nonetheless. This may be due to an increased reliance on innate immunity, and there has been a steady increase in interest in the role of innate immunity in testicular function recently (Com et al. 2003; Bhushan et al. 2008; Starace et al. 2008).

Less intuitive is the fact that immune cells, cytokines, and other immune mediators appear to play a crucial role in normal testicular function. These include a role for the testicular macrophages in regulating Leydig cell proliferation, development, and steroidogenesis and the involvement of inflammatory signaling pathways and cytokines produced by the Sertoli cells in the control of spermatogenic cell development—these topics have been extensively reviewed elsewhere (Hedger and Hales 2006; Hedger 2011). This close relationship between innate immunity in the testis and normal testis function has consequences for fertility and provides an explanation for the observation that steroidogenesis and sperm production can be compromised by systemic infections and inflammatory disease (Dong et al. 1992; Baker 1998).

Finally, as previously mentioned, autoimmune infertility leading to testicular damage appears to be a problem for a small subset of men. Presumably, this is due to underlying genetics of susceptibility and is associated with a precipitating event, such as trauma to the reproductive tract or infection. The ability to predict, prevent,

and/or treat this condition will depend upon much better understanding of the unique immunobiology of the testis. It is increasingly evident that failure of these testicular mechanisms underlies male infertility, chronic inflammatory disease, and reproductive tract pain. More generally, uncovering the details of these mechanisms may lead to greater understanding of systemic immunity and tolerance in peripheral tissues and, potentially, for the development of novel immunosuppressive therapeutics.

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