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11.10 Immunology of the Testis and Male Reproductive Tract

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Abbreviations

16:0a-LPC 1-palmitoyl-sn-glycero-3-phosphocholine
18:1a-LPC 1-oleoyl-sn-glycero-3-phosphocholine
18:2a-LPC 1-linoleoyl-sn-glycero-3-phosphocholine
20:4a-LPC 1-arachidonyl-sn-glycero-3-phosphocholine
AID acquired immune deviation
11.10.1 Background

In the decade since this chapter was first published (Hedger 1997), there has been considerable progress in the understanding of the regulation of immunity. Advances such as the emergence of the innate immune system as a major theme in immunity following the discovery of the toll-like receptor (TLR) family (O’Neill and Dinarello 2000), the formalization of multiple macrophage phenotypes
corresponding to the type 1 and 2 helper T (Th) cell subsets (Mantovani et al. 2005), the rehabilitation of suppressor and regulatory T cells (Piccirillo and Thornton 2004), and the identification of new lymphocyte subsets, including Th3, Th17, and natural killer T (NKT) cells (Godfrey et al. 2000; MacDonald 1998; Romagnani 2008), also have had considerable influence on the concept concerning the immunology of the male reproductive tract. Nonetheless, while there have been advances in our understanding of the interface between the male reproductive tract and the immune system, there is still much we simply do not know. The need to understand this interface and the potential implications for reproductive toxicology continues to be just as important as ever.

There is no doubt that the testis and the male germ cells in particular are susceptible to immunological damage. Clinically significant testicular autoimmunity, most commonly indicated by the presence of antisperm antibodies in the serum or ejaculate, is implicated in the case of 5–12% of all male infertility patients within the developed world (Baker et al. 1983; Pattinson and Mortimer 1987), but the incidence is much higher in populations where access to reproductive health care is limited (Ekwere 1995). Common factors suspected to contribute to testicular autoimmunity include reproductive tract infections, physical trauma, immune system dysfunction, and genetics. The release of spermatogenic antigens from the reproductive tract following vasectomy generally results in autoimmune responses, ranging in severity from sperm antibodies to orchitis, in both humans and experimental animals (Alexander and Anderson 1979; Flickinger et al. 1990a; Kojima and Spencer 1983; Tung and Alexander 1980). The potential for spontaneous testicular autoimmune disease in otherwise normal men remains poorly characterized, although studies in animals clearly indicate the possibility of such reactions in humans (Furbeth et al. 1989; Tung et al. 1981). Complicating matters is the fact that the testis is an immunologically privileged tissue, in the sense that foreign tissue grafts into the testes of experimental animals survive for prolonged periods (Barker and Billingham 1977; Head et al. 1983a; Whitmore and Gittes 1978). This dichotomy between immune susceptibility and privilege is indicative of a highly specialized interaction between the male reproductive system and the immune system, with potentially important implications for both systems. The objective of this chapter is to provide an overview of the current state of knowledge and to highlight issues for further consideration that may be of relevance to male reproductive toxicology.

### 11.10.2 The Interface between the Immune System and the Reproductive Tract

The immune system is the body’s protective arsenal against disease. It operates via the detection of potential aggressors, recognized through conserved motifs found on pathogenic organisms (innate immunity) or confrontation by completely novel (i.e., foreign) molecular structures (adaptive immunity) (Vivier and Malissen 2005; Zinkernagel 2000). Immunity involves specialized cells possessing highly developed recognition and activation abilities (macrophages and dendritic cells) and the cells that carry out the protective responses: T and B cells in the case of adaptive immunity and NK cells and mononuclear or polymorphonuclear phagocytes (PMNs) in the case of innate immunity. Many of these cells play roles in both the recognition and effector arms of the immune response and in both innate and adaptive immunities (Figure 1). Innate immunity provides the rapid response elements of the immune system, but is limited in its attack repertoire. Adaptive immunity is much more flexible in its responses, but requires some time to become effective. Once the adaptive arm has been activated toward a particular pathogen, however, it can respond rapidly in the future due to the persistence of memory lymphocytes, which form the basis of immunization (McGhee et al. 1993; Schittek and Rajewsky 1990).

#### 11.10.2.1 The Innate Immune Response

The innate immune system operates upon recognition by specific pattern-recognition receptors of specific motifs found on pathogenic organisms (innate immunity) or confronting by completely novel (i.e., foreign) molecular structures (adaptive immunity) (Vivier and Malissen 2005; Zinkernagel 2000). Functionally, these receptors have evolved to recognize pathogen-associated molecular patterns (PAMPs), such as bacterial lipopolysaccharides (LPSs), lipoproteins, peptidoglycans, and viral nucleic acids (Bianchi 2007; Roach et al. 2005). Unlike classical ligand receptors, these receptors respond to multiple ligands possessing related, but not necessarily identical, structures. The largest
and best-studied class of pattern-recognition receptors is the TLRs, which are members of a larger family of proteins that include the interleukin (IL) 1 receptor (IL1R), and which share a conserved cytoplasmic domain called the Toll/IL1R (TIR) domain (Akira and Takeda 2004; O’Neill and Bowie 2007). In humans, 10 functional TLRs (TLR1–10) have been identified (Figure 2). Mammalian TLRs 11–13 are known from laboratory rodent species, but are either pseudogenes or absent in humans (Roach et al. 2005). Although they are particularly associated with cells of the monocyte lineage (i.e., macrophages and dendritic cells), these receptors are also found on certain T and B cells, as well as many fibroblastic and epithelial cells, including those of the male urogenital tract (Lauw et al. 2005; Zhang et al. 2004). Significantly, ligands for some of the TLRs include endogenous molecules of mammalian origin (Barrat et al. 2005; Karikó et al. 2004; Tsan and Gao 2004; Yasuda et al. 2005; Yu et al. 2006). TLR signaling occurs through engagement of crucial adaptor proteins, specifically myeloid differentiation primary response protein 88 (MyD88) or TIR domain-containing adaptor protein inducing interferon β (TRIF), via TIR domain interactions, leading to activation of tumor necrosis factor (TNF) receptor-associated factor 3 and 6 (TRAF3, TRAF6) (Akira and Takeda 2004; O’Neill and Bowie 2007; O’Neill and Dinarello 2000). Based on the TLR engaged, this results in nuclear translocation of the inflammatory transcription factor, nuclear factor kappa B (NFκB), activation of the Jun N-terminal kinase (Jnk) and p38 mitogen-activated protein (MAP) kinases, and induction of type 1 interferon (IFNα and IFNβ).
The pattern-recognition receptor family also includes the nucleotide binding and oligomerization domain (Nod)-like receptor (NLR) family and the retinoic acid-inducible gene (RIG)-like helicases (RLHs) (Becker and O’Neill 2007; Thompson and Locarnini 2007). The NLRs detect various bacterial PAMPs within the cytosol. Some NLRs, such as Nod1 and Nod2, act via NfκB, while other NLRs work through induction of the cysteine protease caspase 1, which activates the proinflammatory cytokines IL1β and IL18 and initiates caspase-mediated proapoptotic pathways within the target cell. The RLHs, which detect the presence of viral RNA in the cytosol, activate both NfκB and production of type 1 IFNs.

Activation of innate immunity leads to inflammation, characterized by increased blood and lymphatic flow within the affected tissue and recruitment of immune cells to the site through production of chemokines and expression of vascular leukocyte adhesion molecules (Moser and Loetscher 2001; Picker and Butcher 1992). This mobilization of immune cells is accompanied by activation of complement, production of proinflammatory cytokines...
and acute-phase proteins, and stimulation of phagocytosis and killing of pathogens and infected cells by macrophages, PMNs, and NK cells, through production of proteases and other lytic proteins, and cytotoxic reactive oxygen species (ROS) and induction of apoptotic pathways (Rosenberg and Gallin 2003). Moreover, activation of innate immunity is responsible for initiation of the adaptive immune response, through production of regulatory cytokines and stimulation of the antigen-presenting cell (APC) abilities of dendritic cells, macrophages, and some B cells (Kelsall and Rescigno 2004; Spörri and Reis e Sousa 2005).

Among the cell types that mediate the innate immune response, NK cells represent a special case. These cells are of the lymphocytic lineage, but they are capable of spontaneously attacking target cells without prior sensitization by exposure to antigen (Mori et al. 2001; Westermann and Palbst 1992). Through a complex of specific receptors on their surface, NK cells are able to recognize and directly target transformed cells, such as virally infected or tumor cells (Lanier 2001; Mori et al. 2001). In this, NK cells provide a first-line of lymphocyte defense within the context of the innate immune response, but activated NK cells also participate in adaptive immunity, both as regulators and as effector cells of the response (Mocikat et al. 2003; Rauler 2004).

11.10.2.2 The Adaptive Immune Response

Highly specific interactions between APCs and T cells, mediated via the polymorphic proteins of the major histocompatibility complex (MHC; the human leukocyte antigen (HLA) complex in humans) and antigen-specific T cell receptors (TCRs), are crucial to the adaptive immune response. The APCs process exogenous foreign proteins into short antigenic peptides, which are then incorporated into a structural groove on the external surface of the MHC protein complex during its assembly within the cell (Cresswell et al. 1999; Sant et al. 1999). The TCR on the surface of a T cell that has specificity for the antigen binds to the antigen–MHC complex on the APC surface, which results in activation and proliferation of the T cell. Circulating T cells express one of two coreceptors, CD4 and CD8, as part of the TCR, which direct them to recognize antigens associated with either MHC class II or I proteins, respectively (Janeway 1992). Antigens are presented to CD4+ T cells by the so-called professional APCs that express MHC class II antigens (i.e., dendritic cells, macrophages, and B cells) (Heath and Carbone 2001; Unanue 1984). The CD8+ T cells recognize antigens presented in the context of MHC class I proteins, which are expressed by almost all cell types in the body. Activation of the T cell also requires physical interaction between the APC and T cell involving costimulatory ligand–receptor pairs, particularly CD8: B7 and CD40:CD40L, as well as the production of either type 1 cytokines (IFNγ, IL2, and IL12) or type 2 cytokines (IL4, IL5, IL10, and IL13) (Constant and Bottomly 1997; Heath and Carbone 2001; Moser and Murphy 2000). As a consequence, the activated T cell can have a number of different fates depending on the costimulatory molecules engaged and cytokines produced: activated CD4+ T cells may become type 1 helper (Th1) cells, which direct development of the cellular immune response involving cytotoxic CD8+ T cells, or they may become type 2 helper (Th2) cells, which promote the development of B cells into antibody-secreting plasma cells following interaction with their antigen (DeFranco 1987). Recently, a new Th effector cell subset (Th17) developmentally related to Th1 cells, but producing IL17, IL21, and IL22 has been described (Ouyang et al. 2008; Romagnani 2008). These Th17 cells direct a number of responses involved in protection against extracellular pathogens, including recruitment and activation of the major circulating PMN subset, the neutrophils, differentiation of B cells, and inflammatory reactions of epithelial cells. However, this cell subset has also been implicated in the development of autoimmune diseases and, along with Th2 cells, in allergy (Oboki et al. 2008; Ouyang et al. 2008). The absence of appropriate costimulatory molecule interactions and/or the presence of specific regulatory cytokines may also lead to T cell inactivation and deletion or generation of regulatory (i.e., suppressor) T cell subsets (Gilliet and Liu 2002; Nossal 1994; Piccirillo and Thornton 2004; Thompson and Thomas 2002). Finally, at least some activated T and B cell clones persist as memory cells following the resolution of the immune response (Dutton et al. 1998; Schittek and Rajewskey 1990).

The initial antigen presentation and lymphocyte activation events generally occur in the secondary immune tissues, the local lymph nodes and spleen. Consequently, an effective lymphatic drainage is usually necessary for immune responses to develop in a particular tissue. However, since leukocytes recirculate through tissues, many of the important events, including antigen exposure and processing,
aggregation recognition by antigen-exposed lymphocytes, and lymphocyte maturation and proliferation, also occur at the reaction site itself (Ascher et al. 1983; Gruber 1992; Pedersen and Morris 1970). Moreover, while it is generally accepted that the adaptive immune system is activated by the presence of foreign antigens, there is an alternative activation model that combines elements of both the innate and adaptive immune systems. This is encapsulated by the ‘danger hypothesis,’ which proposes that APCs respond to substances that cause or signal damage, rather than to those that are simply foreign (Matzinger 1994). These danger signals include CD40L, the early proinflammatory cytokines IL1β and TNFα, IFNs, and heat-shock proteins as well as substances that are normally found only inside cells (e.g., nucleotides, unmethylated CpG sequences in mammalian double-stranded DNA) and hyaluron breakdown products (Gallucci and Matzinger 2001). In this model, activation of the immune system occurs as a response to evidence of an extant threat, rather than toward a specific feature of the threat itself. This mechanism may contribute to the onset of certain autoimmune diseases.

### 11.10.3 Immunological Tolerance

While an effective immune response is essential for protection of the host from a range of threats, the immune system also has its dark side. The capacity of the immune system for destructiveness is expressed in chronic inflammatory and autoimmune diseases, including those that affect the male reproductive tract (Roper et al. 1998; Schuppe and Meinhardt 2005; Schuppe et al. 2008; Suominen 1995). These examples of an immune system seemingly out of control highlight the crucial importance of effective immunoregulation. At the center of immunoregulation is the concept of tolerance, or the capacity of the immune system to ignore certain molecular patterns, and the systems that restrain the immune response once its usefulness has expired.

Tolerance is the ability of the immune system to discriminate self from nonself. It is fundamentally based on the removal, inactivation, or suppression of self-reactive lymphocyte subsets, so that the immune system becomes effectively incapable of recognizing and reacting to these antigens. Induction of tolerance comprises both central and peripheral mechanisms. The central mechanism involves clonal deletion of T and B cell subsets that are potentially autoreactive, a process that normally occurs during gestation as part of the early maturation of the immune system (Kappler et al. 1987; Nossal 1994). Many details of this editing process remain incompletely understood, although the process involves broad expression by thymic medullary cells of self-antigens, including many endocrine tissue-specific antigens under the control of the transcription factor autoimmune regulator (Aire) (Liston et al. 2003). Lymphocytes that recognize these self-antigens within the thymus during this process are targeted for destruction, effectively removing them from the lymphocyte repertoire. In addition, there are a number of complex mechanisms of peripheral tolerance, which involve the inactivation of antigen-specific lymphocytes in peripheral lymphoid organs by weak antigen stimulation in the absence of appropriate costimulation and the production of lymphocytes with immunoregulatory activities (Gilliet and Liu 2002; Piccirillo and Thornton 2004; Thompson and Thomas 2002). Peripheral immunoregulation involves several antigen-specific regulatory or suppressor T cell types, most notably the CD4⁺CD25⁺ regulatory T cell (Treg) subset. These cells selectively produce the immunosuppressive cytokines transforming growth factor β (TGFβ) and IL10, but also appear to act via direct contact with the cell being suppressed (Nakamura et al. 2001; Piccirillo et al. 2002). Other T cell subsets implicated in immunosuppression through their ability to produce TGFβ and IL10 include Th3 cells, Tr1 (T regulatory 1) cells, and γδ T cells, the latter being a minor T cell subset that is generally associated with epithelia (Kuhlein et al. 1994; MacDonald 1998; Seo et al. 2001). The NKT cells, which are T cells with NK activity that possess unique restriction to glycolipid antigens presented by the MHC-like molecule CD1d, play a role in promoting graft survival and development of CD8⁺ regulatory/suppressor T cells through production of IL4 and IL10 (Godfrey et al. 2000; Nakamura et al. 2003; Sonoda et al. 2001). The frontline NK cells are able to modulate adaptive immune responses by inducing dendritic cell death through contact-mediated lysis, but are also capable of producing IFNγ to stimulate the antigen-presenting activity of dendritic cells (Laouar et al. 2005; Raulet 2004).

It is the absence of tolerance that inhibits graft success in the very artificial condition of tissue transplantation. The immune cells of both the graft recipient and the donor tissue respond to one
another, particularly with respect to polymorphic differences of the MHC proteins, leading to rejection of tissues that are not antigenically matched (Gould and Auchincloss 1999). Even under normal conditions, failure of preexisting tolerance may occur through a number of mechanisms, leading to autoimmune disease. Somatic mutation of the antigen receptor expressed by a T or B cell may result in the creation of new self-reactive clones, subverting central tolerance (Burrows et al. 2000). Autoimmunity may also occur when the inflammatory response to an infection damages or overwhelms normal mechanisms of self-tolerance, or where the infection involves organisms that express antigens that may cross-react with self-antigens (molecular mimicry) (Merkler et al. 2006; Regner and Lambert 2001).

In addition to lymphocyte-mediated tolerance already discussed, the immune system possesses a repertoire of mechanisms that control and limit inflammatory and immune responses once they have commenced. Inflammation triggers the production by the adrenal gland of corticosteroids, which inhibit the production of proinflammatory cytokines and other immune mediators, reduce the expression of leukocyte adhesion molecules, and induce lymphocyte apoptosis (Buckingham et al. 1996; Kapcala et al. 1995). During inflammation, immune cells themselves produce anti-inflammatory and immunosuppressive molecules, including prostaglandins D and J and the lipoxins (Herlong and Scott 2006; Levy et al. 2001). Moreover, activated lymphocytes have a limited lifespan, undergoing a process of activation-induced cell death following upregulation of the extrinsic apoptotic signal mediated by interaction of the Fas receptor (CD95) with its ligand (Fas ligand: FasL or CD95L), eventually leaving behind a relatively small number of long-lived memory cells (Dutton et al. 1998; Schittek and Rajewsky 1990). Likewise, there are mechanisms to inhibit antibody activity and promote antibody clearance (Isaacs 1990).

### 11.10.3.1 The Concept of Immune Privilege

Classically, the term immune privilege applies to organs or tissues where survival of foreign grafts may be prolonged (Barker and Billingham 1977), but more accurately, the term denotes tissues where immune responses are inhibited or suppressed (Hedger 2007). Privileged tissues include the brain, eye, pregnant uterus, and the testis. Initially, privilege was attributed to deficient lymphatic drainage in such tissues, or physical barriers that prevented immune cells from gaining access (Barker and Billingham 1977). Several privileged tissues do possess highly specialized epithelial or endothelial cell barriers that may sequester antigens away from the normal immune surveillance: the best characterized of these are the blood–brain barrier and the trophoblast in the pregnant uterus (Hunt 2006; Streilein 1993). However, physical exclusion is now considered an overly simplistic explanation for immune privilege, given that lymphocyte and antibody responses to introduced antigens occur even in sites where grafts can survive for extended periods (Head et al. 1983b; Kaplan and Streilein 1978; Tafuri et al. 1995). Although barrier mechanisms and altered lymphatics may be contributory factors in some tissues, immune privilege is a consequence of tissue-specific specialized systems for controlling, subverting, or preventing local inflammatory or immune activation responses (Hedger 2007).

### 11.10.3.2 Testicular Organization and Immunity

The male reproductive tract comprises the paired testes, associated epididymis and vas deferentia, accessory glands (chiefly the prostate and seminal vesicles), and the urethra. These tissues represent a diversity of structures and, consequently, the immunology of each tissue retains distinctive features (Hedger and Hales 2006). Best studied in this context is the testis, which comprises two discrete tissue compartments, the seminiferous tubules and the interstitial tissue. The interstitial tissue contains the blood supply and lymphatics, as well as the innervation of the testis (Fawcett et al. 1973; Setchell et al. 1994). The epithelium of the seminiferous tubules is entirely avascular. Within the seminiferous epithelium, spermatogenesis is supported and maintained by the Sertoli cells, which remain in physical contact with the developing germ cells at all times. During spermatogenesis, a stem cell spermatogonium sitting on the basement membrane of the seminiferous tubule becomes committed to a series of mitotic divisions, producing a cohort of spermatocytes. The spermatocytes subsequently move toward the tubule lumen, passing through tight junctions between adjacent Sertoli cells, into the highly specialized environment of the adluminal compartment (Wang and Cheng 2007). Within this compartment, the spermatocytes divide meiotically to produce haploid spermatids and then undergo
differentiation into spermatozoa, which are ultimately released into the lumen of the tubule leaving behind the majority of their cytoplasm to be digested by the Sertoli cells as residual bodies. The released spermatozoa are swept by fluid secreted from the Sertoli cells toward the rete testis, a collecting structure lined by a simple epithelium that is connected to the adjacent epididymis by a series of efferent ducts. Sperm mature and are stored in the epididymis, until they are either released through the vas deferentia at the time of ejaculation or removed by epididymal phagocytes (Barratt and Cohen 1987; Roussel et al. 1967).

The process of spermatogenesis is highly organized, with multiple generations of developing germ cells in each region of the epithelium maintaining distinct, progressing cellular associations or stages, which comprise the cycle of the seminiferous epithelium (14 stages in the rat, 12 stages in the mouse, 6 stages in man). These stages are arranged in sequence along the length of each seminiferous tubule, creating waves of coordinated spermatogenic development (Clermont 1972). Spermatogenesis is maintained and controlled by follicle-stimulating hormone (FSH) and testosterone, the latter being produced by the Leydig cells in the interstitial tissue under the influence of luteinizing hormone (LH). Importantly, FSH and testosterone act upon Sertoli cells and not directly on the germ cells (McLachlan et al. 1996). These hormones determine the efficiency of the spermatogenic process largely by controlling germ cell survival, that is, the balance between differentiation and apoptosis (Matthiesson et al. 2006; Ruwanpura et al. 2008). In order to maintain the cycle of the seminiferous epithelium, the developing germ cells and Sertoli cells are engaged in continuous intercellular communication, although the precise nature of this communication remains very poorly understood.

The testis, and spermatogenesis in particular, presents a unique challenge for the immune system. Although there are reports of direct drainage of some lymphatic vessels to the thoracic duct, there is no evidence of any structural or functional deficiency in the efferent lymphatics of the testis, and allogeneic cells injected into the rat testis induce typical immune responses in the draining lymph nodes (Fawcett et al. 1973; Head et al. 1983b; Itoh et al. 1998a; Moller 1980). Nonetheless, the vast majority of spermatogenic differentiation occurs long after central tolerance is established and, while thymic expression of some testicular antigens is under the control of the Aire transcription factor, it is obvious from clinical and experimental studies that germ cells express multiple autoreactive antigens and are highly immunogenic when brought into contact with the immune system (Itoh et al. 1991; Tung 1975). Surprisingly, this is a problem for a relatively small cohort of men only, and autoimmune orchitis or epididymitis is rarely reported in humans, except in the context of local infection (Krieger 1984). Even allowing for the possibility that most cases of spontaneous autoimmune reactions against the developing germ cells are never diagnosed, since they would occur early in reproductive life, and would simply present as spermatogenic failure during infertility work-ups (Schuppe and Meinhardt 2005; Schuppe et al. 2008), why are such reactions not more common?

11.10.3.3 The Role of the Blood–Testis Barrier and Immune Ignorance

It is widely believed that the blood–testis barrier plays a role in protecting testicular antigens from the immune system. However, as a result of a common misunderstanding of the nature of this vitally important structure, its actual role is usually overestimated. Critically, the blood–testis barrier involves highly specialized occluding or tight junctions between adjacent Sertoli cells, which separate the spermatogonial and early meiotic cells from the majority of meiotic and postmeiotic germ cells (Dym and Fawcett 1970; Setchell et al. 1969). The structure and functions of the barrier do not need to be discussed in detail here, as this topic is covered in much more detail in other chapters in this volume (see Chapter 11.05). By preventing the passage of most molecules between adjacent Sertoli cells, the blood–testis barrier allows the creation of a highly specialized biochemical environment essential for meiotic development (Cheng and Mruk 2002; Griswold 1988). It also blocks access by lymphocytes, complement, and antibody (Ben et al. 1986; Yule et al. 1988). By contrast, the vascular endothelium and peritubular cells surrounding the tubules play a negligible role in maintaining the blood–testis barrier in practical terms, and immune cells and proteins have relatively free access to the interstitium and basal seminiferous epithelium (Mahi-Brown et al. 1988; Yule et al. 1988). Thus, this barrier is quite different in properties and function from the blood–brain barrier, located on the vascular endothelium, or the trophoblastic barrier of pregnancy, which entirely
segregates the developing fetal tissues from the maternal host.

A reinterpretation of the blood–testis barrier as a series of cellular elements that protects the germ cells from harmful influences has been proposed more recently (Bart et al. 2002). In this model, the blood–testis barrier consists not only of the structural elements already discussed, but also the efflux pump barrier system involving the drug transport proteins 

\[ p \]-glycoprotein and the multidrug resistance–associated protein-1 expressed on the capillary endothelium, peritubular myoid cells, and the basal aspect of the Sertoli cells (Bart et al. 2004; Melaine et al. 2002). While these elements may certainly be important for protection of the testis against toxic agents, their contribution to immunoregulation is probably marginal.

Several lines of evidence indicate that the blood–testis barrier cannot account for all manifestations of immune privilege in the testis. The barrier does not prevent exposure of germ cell antigens to the immune system, as antibodies and lymphocytes specific for spermatogenic antigens appear to be a normal feature of the circulating immune repertoire even under normal conditions (Turek and Lipshultz 1994). Spermatogenic cell autoantigens are not confined behind the Sertoli cell tight junctions, and are expressed by spermatogonia and the early spermatocytes (Mahi-Brown et al. 1987; 1988; Yule et al. 1988). Moreover, the barrier does not extend into the rete testis or beyond. Significantly, orchitis can be passively transferred to naive mice using lymphocytes obtained from mice with active autoimmune orchitis, with the initial reaction concentrated in the interstitial tissue around the rete testis (Mahi-Brown et al. 1987; Tung et al. 1987). A similar initial pattern of development of orchitis within the rete testis region has been observed in mice actively immunized with viable germ cells (Itoh et al. 1995a). In many seasonally breeding species, annual regression of the blood–testis barrier occurs without inducing overt inflammation or autoimmunity (Pelletier 1986; Tung et al. 1981). Finally, the blood–testis barrier cannot explain the enhanced survival of grafts within the interstitial tissue (i.e., outside the barrier) and does not explain survival of autoantigenic sperm in the epididymis, or remainder of the male reproductive tract. However, while the blood–testis barrier does not explain immune privilege in the testis, the breach of the tight junctions comprising the barrier remains a critical event in the development of any autoimmune destruction of the seminiferous epithelium during orchitis (Mahi-Brown and Tung 1989; Mahi-Brown et al. 1987).

Recognition of antigen in association with MHC is essential to normal adaptive immune responses, and reduced expression of MHC class I (HLA-A, HLA-B, and HLA-C in humans) and class II proteins (HLA-D) appears to be an important feature of immune-privileged tissues and tumors (Garrido et al. 1997; Haas et al. 1988; Head andBillingham 1985; Wekerle et al. 1987). Reduction in class I and II expression reduces the likelihood of immune-activating events involving CD4+ helper T cells and cytotoxic CD8+ T cells in the tissues. Studies indicate a characteristic absence of expression of MHC proteins on the cells of the seminiferous epithelium under normal conditions (Haas et al. 1988; Head andBillingham 1985; Lustig et al. 1993; Tung et al. 1987), although both MHC classes are expressed in human spermatooza (Martin-Villa et al. 1996). There is evidence from studies on mouse Sertoli cells in culture that MHC class II expression can be upregulated in these cells by IFNγ (Dal Secco et al. 2008). In contrast to the seminiferous epithelium, both MHC class I and II proteins are expressed throughout the testicular interstitial tissue: MHC class I is expressed on most interstitial cells, including the Leydig cells (Haas et al. 1988; Pöllänen and Maddocks 1988), while testicular macrophages and dendritic cells express MHC class II (Haas et al. 1988; Head andBillingham 1985; Hedger and Eddy 1987; Mahi-Brown et al. 1987; Tung et al. 1987). Thus, it appears unlikely that a lack of APCs is a contributing factor in testicular immune privilege, although differences in the number and distribution of MHC class II-expressing cells in the interstitial tissue of different species or strains may help to explain differences in susceptibility to autoimmune orchitis (Flickinger et al. 1990b; Kojima and Spencer 1983; Teuscher et al. 1987).

The fact that the testis is not isolated from the immune system, yet can tolerate both endogenous (auto-) antigens associated with spermatogenesis and exogenous (allo- or xeno-) antigens expressed by grafts, is evidence of tissue-specific inhibition of the immune response. The question then arises: if immune responses against exogenous and endogenous antigens are inhibited in the testis environment, does the testis display a reduced capacity to deal with infections and greater susceptibility to certain kinds of tumors? There is evidence that immunity may be compromised in some situations, for example, the tendency toward relapsing acute lymphocytic leukemia in the testes (Hudson et al. 1985), and several
systemic viral and mycobacterial infections target the testis, including mumps, human immunodeficiency virus, tuberculosis, and the severe acute respiratory syndrome virus (Krieger 1984; Nistal et al. 1986; Xu et al. 2006). However, the testis does not appear to be immunodeficient. Even in comparison with the rest of the male reproductive tract, orchitis due to ascending infections is relatively rare (Krieger 1984; Ness et al. 1997). Moreover, the extremely variable level of success of experiments by different groups using different transplant models has shown that immune privilege of the testis is both limited and conditional (Dobrinski 2005; Head et al. 1983a; Maddocks and Setchell 1988; Selawry and Whittington 1984; Setchell et al. 1995). Just what are these necessary conditions, however, remains poorly defined. Studies indicate that the effector arm of the immune response is intact within the testis, and it is the ability to recognize and react toward foreign antigens and/or mount a normal inflammatory response that is suppressed (Head and Billingham 1985; Head et al. 1983a; Mahi-Brown and Tung 1989; Mahi-Brown et al. 1987).

In summary, there appears to be a potentially unique functional interaction between the testis and the immune system, which involves control of the mechanisms normally involved in immune activation, in order to limit adaptive immune responses without drastically compromising the ability of the testis to protect itself when required.

### 11.10.3.4 Testicular Leukocytes

Although their presence and influence is almost universally neglected, macrophages, lymphocytes, and PMNs have varying degrees of access to the interstitial tissue of the testis (Table 1). They are generally excluded from the seminiferous epithelium under normal conditions. Over the past 10 years, studies have started to fill in some of the details concerning these cells.

#### 11.10.3.4.1 Macrophages

Macrophages are by far the most prominent immune cells in the testis. They are found in the testes of all mammalian species so far examined, but are particularly numerous in human, rat, and mouse testes (Frungieri et al. 2002; Hume et al. 1984; Mendis-Handagama et al. 1987; Miller et al. 1984; Vergouwen et al. 1993; Wang et al. 1994). Typically, macrophages arise from myeloid precursors in the bone marrow and circulate in the blood as monocytes. They are capable of adopting a broad range of structural and functional phenotypes that are largely dictated by the tissues in which they become resident (Mantovani et al. 2005; van Furth 1988). Characteristically, they are robustly phagocytic and possess a potent arsenal of antimicrobial and cytotoxic activities, such as the ability to generate large amounts of ROS. They produce many immunoregulatory products, including cytokines, eicosanoids (prostaglandins, thromboxanes, leukotrienes, and lipoxins), and nitric oxide, all of which are important in establishing and controlling the inflammatory process, but also have important roles in tissue remodeling, healing, and normal homeostasis. All macrophage lineages can express MHC class II antigens and have the capacity to present to CD4+ helper or Treg cells (Unanue 1984). Thus, macrophages are the cells that link innate and adaptive immunity, and dendritic cells are a highly specialized macrophage subset that is particularly effective in this role (Heath and Carbone 2001).

| Cell type          | Characteristics          | Local functions                                      |
|--------------------|--------------------------|------------------------------------------------------|
| Monocytes/macrophages | Numerous, multiple subsets | Phagocytosis, tissue remodeling, Leydig cell development, innate immunity (antimicrobial), immunoregulation |
| Dendritic cells    | Present                  | Antigen presentation, immunoregulation               |
| T cells            | CD4+, CD8+, and NK T subsets | Adaptive immunity, immunosurveillance, immunoregulation |
| NK cells           | Present                  | Innate immunity, immunosurveillance, immunoregulation |
| Mast cells         | Species-specific distribution | Vascular control, innate immunity                      |
| Eosinophils        | Species-specific distribution | Vascular control, innate immunity                      |
| Neutrophils        | Not normally present     | Inflammation, phagocytosis, innate immunity (antimicrobial) |
Testicular macrophages have been most extensively studied in the rat, with a few studies also in the mouse and human. Studies in the rat indicate that they are heterogenous in their morphology and functions (Bryniarski et al. 2004; Gerdprasert et al. 2002a; Itoh et al. 1995b; Wang et al. 1994). Their absolute numbers are primarily under Leydig cell control, but evidence suggests that FSH acting via the Sertoli cell has a role in regulating their functional activity (Duckett et al. 1997a,b; Gaytan et al. 1994c; Wang et al. 1994). Macrophages accumulate within the interstitial tissue at the time of puberty, and play critical roles in the normal development of the testis, particularly by promoting the proliferation and maturation of the Leydig cell population (Cohen et al. 1997; Gaytan et al. 1994a,b; Hardy et al. 1989; Itoh et al. 1999; Raburn et al. 1993; Vergouwen et al. 1993). Through their ability to produce a broad array of growth factors, prostanoids, and vasoactive regulators, other developmental and homeostatic roles within the testis may be predicted as well.

Data concerning the immunological functions of testicular macrophages are based on a relatively small number of studies in either the rat or the mouse, and are patchy at best. Indeed, there is some evidence for distinct differences even between these two closely related species. In general, testicular macrophages have intact phagocytic, cytotoxic, and antimicrobial functions (Miller et al. 1984; Wei et al. 1988). Rat testis macrophages express MHC class II antigens (Head and Billingham 1985; Hedger and Eddy 1987; Wang et al. 1994), but display a reduced capacity for lymphocyte activation in vitro (Kern and Maddocks 1995). Testicular macrophages in the human and mouse also express MHC class II, although expression in the mouse appears to be more restricted (Haas et al. 1988; Itoh et al. 1995b; Pöllänen and Niemi 1987; Tung et al. 1987). A lack of expression of the essential costimulatory molecules, B7-1 (CD80) and B7-2 (CD86), has been reported in the mouse testis as well (Sainio-Pöllänen et al. 1996). Studies also indicate that macrophages from the rat testis have a significantly reduced ability to produce proinflammatory cytokines, such as IL1β and TNFα, following activation (Gerdprasert et al. 2002a; Hayes et al. 1996; Kern and Maddocks 1995; Kern et al. 1995; Meinhardt et al. 1996), and stimulated mouse testicular macrophages produce the anti-inflammatory/immunosuppressive cytokines IL10 and TGFβ, in vitro (Bryniarski et al. 2004, 2005). While the data suggest that testicular macrophages may have a predominantly anti-inflammatory and/or immunosuppressive phenotype, this has yet to be formally established. Furthermore, dendritic cells, which are a highly specialized macrophage subset also found within the testicular interstitium (Fijak et al. 2005; Hoek et al. 1997; Itoh et al. 1995b; Rival et al. 2006a), presumably play a more important role in controlling immune responses to antigens within the testis (Fijak et al. 2005; Rival et al. 2006a, 2007).

In the rat testis, macrophages can be discriminated by their differential expression of two molecular markers recognized by the antibodies ED1 and ED2. Antibody ED1 recognizes the lysosomal protein CD68, which is associated with antigen processing and presentation (Grosman et al. 2002), while ED2 recognizes the scavenger receptor CD163 (Fabriek et al. 2005). CD163 is involved in the clearance of hemoglobin:hapten proteins and the resolution of inflammation (Philipidis et al. 2004). About 80–85% of rat testicular macrophages express this scavenger receptor and about half of these cells also lack expression of CD68 (Gerdprasert et al. 2002a; Meinhardt et al. 1998; Wang et al. 1994). In the human testis, most macrophages appear to be CD68+, but expression of CD163 is a feature of a subset of these cells as well (Frungieri et al. 2002). The heterogeneous expression of these two markers points to the existence of multiple populations of testicular macrophages, and evidence suggests that these phenotypic differences also correspond to functional differences. In the normal and inflamed adult rat testis, CD163+ cells lack expression of the key inflammatory mediators IL1β and inducible nitric oxide synthase (iNOS), as measured by double-label immunohistochemistry, but these molecules are detectable in CD68+ cells that do not express CD163 (Gerdprasert et al. 2002a; O’Bryan et al. 2005). Indeed, purification of CD163+ macrophages from the rat testis using immunomagnetic beads confirms that these cells have reduced capacity to produce IL1β, IL6, or NO upon stimulation, although they retain the ability to produce prostaglandins (Gerdprasert et al. 2002a; Hedger, unpublished data). This suggests that the CD163+ macrophages have reduced proinflammatory activities, while the CD163-negative testicular macrophage population retains normal proinflammatory functions. This observation is important because macrophages that express CD163 and produce IL10, but display reduced expression of CD80, CD86, iNOS, TNFα, and IL1β, are polarized toward the immunoregulatory M2 subset (Mantovani et al. 2005;
Sica et al. 2008). The M2 macrophage subset is analogous to the Th2 subset among T cells, and is associated with late-stage inflammation and its resolution, as well as tumors and other immunologically privileged tissue sites. It would appear that a significant proportion of the resident macrophages of the testis possess an immunoregulatory phenotype that is consistent with immune privilege. The functional roles of this macrophage population, as well as those of the macrophages that appear to retain normal inflammatory (i.e., M1 subset) properties, demand particular attention if we are to understand the unique immunological environment of the testis.

11.10.3.4.2 Lymphocytes
While the functions and regulation of lymphocytes within the testis have yet to be properly characterized, by their presence alone these cells will have significant effects on testicular events. It may also be assumed that, in contrast to the largely resident population of testicular macrophages, lymphocytes circulate through the testis. This may be because they possess specificity for testicular antigens, or because they have been activated during earlier intratesticular inflammatory or infectious events (Czerkinsky et al. 1999; Springer 1994). Quantitative studies in the rat indicate that, at any given time, lymphocytes are approximately 10% as numerous as the macrophages in the normal testis (Hedger and Meinhardt 2000; Hedger et al. 1998b; Wang et al. 1994). In the rat and mouse, intratesticular lymphocytes express T cell and NK cell markers, and comprise distinct T cell, NK cell, and NKT cell subsets (Tompkins et al. 1998; Hedger, unpublished data). Both T cell and NK cell markers are expressed by lymphocytes in the human testis as well (Hedger, unpublished data). B cells are not usually observed in normal tissues, and this includes the testis. In the rat, many T cells possess a memory phenotype, as would be expected (Hedger et al. 1998b; Tompkins et al. 1998). Reanalysis of the data for individual animals across several studies suggests that their numbers tend to be proportional to the numbers of macrophages, but gradually increase with the age of the animal (Hedger et al. 1998b; Hedger and Meinhardt 2000; Wang et al. 1994). Among the T cells of the testis, there is a strong bias toward the CD8+ (i.e., MHC class I restricted, largely cytotoxic) subset (Hedger et al. 1998b; Pöllänen and Niemi 1987; Ritchie et al. 1984; Tompkins et al. 1998; Wang et al. 1994). The prominence of NK cell subsets in the testis is also intriguing given the reduced MHC class I antigen expression in the seminiferous compartment, which would tend to make these cells more susceptible to NK cells (Lanier 2001). Consistent with the anti-inflammatory/immunosuppressive phenotype of the major testicular macrophage subset, T cells and NKT cells in the rat testis constitutively produce IL10 (Hedger, unpublished data). While much remains to be discovered, the existing data concerning the types and activity of testicular lymphocytes are consistent with maintaining immune privilege (i.e., the presence of immunoregulatory lymphocytes) and increased innate immunity (i.e., cytotoxic cell activity).

11.10.3.4.3 Polymorphonuclear Cells
Evidence that PMNs are important in testicular function comes from the fact that mast cells and eosinophils are significant elements of the interstitial tissue in many species. In the rat, mouse, dog, cat, bull, and deer, both cell types are largely absent from the testicular parenchyma, but are associated with blood vessels in the testicular capsule (Anton et al. 1998). Mast cells are found throughout the interstitial tissue in equine and human testes (Anton et al. 1998; Nistal et al. 1984; Yamanaka et al. 2000), and both mast cells and eosinophils are present in porcine species (Anton et al. 1998). The role of these cells in vascular control and innate immunity can be predicted, but they may also play immunoregulatory roles, as mast cells do in other tissues (Galli et al. 2005).

In humans, testicular mast cell numbers are increased in infertility (Meineke et al. 2000; Nagai et al. 1992; Yamanaka et al. 2000), but decline with advancing age (Nistal et al. 1984). The possibility that they accumulate due to past immune events needs to be considered, but they also appear to be actively regulated. In the adult rat testis, mast cell proliferation is under the control of the Leydig cells, suggesting that these cells produce an inhibitor of mast cell activity (Gaytan et al. 1990; Wang et al. 1994). Neonatal estrogen treatment increases mast cell numbers in the rat testis (Gaytan et al. 1989, 1990), and a relationship with elevated Leydig cell aromatase activity is suggested by the prevalence of these cells in boar and equine testes (Eisenhauer et al. 1994; Raeside and Renaud 1983). Neutrophils are found in the testis only under conditions of testicular inflammation or damage (Gerdprasert et al. 2002a; Kohno et al. 1983a; O’Bryan et al. 2000b).
11.10.3.5 Immunity in the Epididymis, Accessory Glands, and Urogenital Tract

The majority of this chapter deals with the testis because it is the primary source of male gametes and hormones, and damage to this organ tends to have more significant consequences for fertility. The remainder of the male reproductive tract comprises the epididymis, vas deferens, accessory glands, and the urethra, which are all epithelial-lined ductal tissues of varying complexity. The immunology of these tissues appears to be quite distinct from that of the testis. While it is true that sperm spend relatively little time within most of these tissues, mature sperm reside within the epididymis for extended periods without provoking an overt immune response, and inflammation and immune cell activity can nonetheless have damaging effects in this organ.

Significantly, these tissues possess normal intercellular junctions, but lack a completely occluding intercellular structure analogous to the blood-testis barrier (Levy and Robaire 1999; Pelletier 2001). Secreted IgA, which lacks the ability to bind complement and possesses principally anti-inflammatory properties as a result, plays an important role in the male urogenital tract (Clifton et al. 1992; Politch et al. 2007; Pudney and Anderson 1995), and the immunology of these organs tends to more closely resemble that of other elements of the mucosal immune system, such as the gastrointestinal and respiratory tracts (Beagley et al. 1998; Clifton et al. 1992; Mestecky et al. 2005). In the absence of the specialized mucosal-associated lymphoepithelial tissue normally found in the gastrointestinal and respiratory tracts, it is likely that the epithelial cells themselves, together with the numerous intraepithelial and stromal macrophages and lymphocytes that are normally found within these tissues, mediate local immunoregulatory mechanisms (Barratt and Cohen 1987; Dym and Romrell 1975; el-Demiry et al. 1985; Ritchie et al. 1984; Pudney and Anderson 1995; Theyer et al. 1992; Yeung et al. 1994). A better understanding of the immunology of these tissues is important. Inflammation, infection, and physical damage can lead to immune reactions against the sperm, which in turn may compromise fertility. Moreover, many men suffer from chronic pelvic pain, generally manifesting as epididymitis or prostatitis in the absence of a detectable infection, a condition that almost certainly involves chronic inflammatory processes within the male reproductive tract.

11.10.4 Immunoregulation in the Male Reproductive Tract

As outlined in the preceding sections, the testis is able to support immune privilege without sacrificing immune surveillance and protection. The mechanisms responsible for this immunological balancing act are still far from understood, but a number of inflammatory and immunoregulatory systems and the processes within the testis have been investigated in varying degrees of detail. It is perhaps not very surprising that many of these regulatory systems also impact upon normal testis physiology, in addition to their immunological and pathological roles.

11.10.4.1 The Immunoregulatory Roles of Testicular Somatic Cells and Germ Cells

Given that the types and properties of leukocytes found within the testis environment are consistent with immunoregulation and enhanced innate immunity, the focus should be on which testicular elements are involved in creating this potentially unique immunological environment. Most attention in this regard has been directed toward the highly versatile Sertoli cell.

Evidence that the Sertoli cell possesses specialized immunoregulatory properties comes from cotransplantation studies. Sertoli cells from immature rat, murine, or porcine testes display extended survival as allografts or xenografts, and cotransplantation of Sertoli cells or testis cell mixtures containing these cells confers increased survival on neural cell xenografts, and pancreatic islet allografts and xenografts (Sanberg et al. 1996; Selawry and Cameron 1993; Suarez-Pinzon et al. 2000). The ability to form a physical barrier through inter-Sertoli tight junctions and the fact that Sertoli cells have reduced MHC expression (Haas et al. 1988; Kohno et al. 1983b; Sanberg et al. 1996; Turek et al. 1996) undoubtedly enhance their potential to avoid T cell activation both in the intact testis and in engraftment studies. Moreover, a soluble form of the nonclassical MHC class Ib molecule HLA-G is produced by the Sertoli cells, as well as spermatocytes, spermatids, and some interstitial cells, in the rhesus monkey testis (Ryan et al. 2002). This molecule has been implicated in apoptosis of alloreactive CD8+ cytotoxic T cells (Fournel et al. 2000). Sertoli cells display lymphocyte-toxic activities and produce molecules with immunosuppressive activity, such as TGFβ1 (De
et al. 1988; Yin 2008; Wu et al. (Bhushan et al.)

TLR mRNA, protein, and/or ligand responses have been observed in testicular macrophages and dendritic cells, immunology. In addition to their predictable expression has been observed in rat Sertoli cells (Bhushan et al. 2008), and low levels of TLR6, 7, and 13 mRNA have been detected in mouse Sertoli cells (Riccioli et al. 2006; Wu et al. 2008). In the rat, germ cells also produce mRNA for TLR2, 3, and 4 in a stage-specific manner, and the peritubular cells express TLR3 and 11, along with low levels of TLR2, 4, and 6 (Bhushan et al. 2008). Rat Leydig cells express TLR2 mRNA and low levels of TLR10 (Bhushan et al. 2008). However, the cytoplasmic receptors TLR8 and 9 have yet to be observed in any nonmyeloid testicular cell type. Of the other pattern-recognition receptors, Nod1 mRNA has been detected in rat Sertoli cells, peritubular cells, and spermatogonia, while Nod2 was detected in spermatogonia only (Bhushan et al. 2008).

Expression of TLRs and other pattern-recognition receptors in the testis is obviously important for responses toward intratesticular pathogens. Infections and inflammation, both systemic and localized, have negative effects on testicular function, resulting in reduced androgen production, lowered sperm counts, and temporary loss of fertility (Adamopoulos et al. 1978; Baker 1998; Carlsen et al. 2003). Presumably, TLRs expressed by the Sertoli cells and germ cells are necessary to detect pathogens that ascend the reproductive tract and, as a consequence, lie behind the blood–testis barrier. This expression within the seminiferous epithelium also suggests a mechanism whereby inflammation can directly inhibit spermatogenesis. Activation of Sertoli cells by TLR ligands induces production of inflammatory mediators, such as IL1α, IL6, and NO, which have direct effects on germ cell development and Leydig cell steroidogenesis (Gérard et al. 1992; Stéphan et al. 1995, 1997; O’Bryan and Hedger 2008; Riccioli et al. 2006; Wu et al. 2008). Moreover, while inhibition of Leydig cell steroidogenesis during inflammation may involve indirect effects at the level of the pituitary, or the actions of inflammatory mediators produced by the testicular macrophages and Sertoli cells, there is evidence that these cells can also respond directly to TLR ligands (Bhushan et al. 2008; Xiong and Hales 1993a).

11.10.4.2 Immunoregulatory Systems in the Testis

11.10.4.2.1 Toll-like receptors

The relatively recent discovery of the TLRs has radically expanded our understanding of innate immunity and promises to do the same for testicular immunology. In addition to their predictable expression on testicular macrophages and dendritic cells, TLR mRNA, protein, and/or ligand responses have been described in several other testicular cells. Rat and mouse Sertoli cells express TLR2, 3, 4, and 5 (Bhushan et al. 2008; Riccioli et al. 2006; Starace et al. 2008; Wu et al. 2008). TLR1, 10, and 11 mRNA expression has been observed in rat Sertoli cells (Bhushan et al. 2008), and low levels of TLR6, 7, and 13 mRNA have been detected in mouse Sertoli cells (Riccioli et al. 2006; Wu et al. 2008). In the rat, germ cells also produce mRNA for TLR2, 3, and 4 in a stage-specific manner, and the peritubular cells express TLR3 and 11, along with low levels of TLR2, 4, and 6 (Bhushan et al. 2008). Rat Leydig cells express TLR2 mRNA and low levels of TLR10 (Bhushan et al. 2008). However, the cytoplasmic receptors TLR8 and 9 have yet to be observed in any nonmyeloid testicular cell type. Of the other pattern-recognition receptors, Nod1 mRNA has been detected in rat Sertoli cells, peritubular cells, and spermatogonia, while Nod2 was detected in spermatogonia only (Bhushan et al. 2008).

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Although the TLRs have evolved to recognize pathogen–derived molecules, such as LPS (TLR4) and bacterial lipoproteins (TLR2), recent data show that they can also be activated by certain endogenous molecules. The ability of preparations of some endogenous molecules, such as heat-shock proteins, to activate TLR signaling is now attributed to contamination of these preparations by bacterial products (Tsan and Gao 2004), but there is convincing evidence that mammalian mRNA is a ligand for TLR3
(Karikó et al. 2004) and mammalian CpG DNA can activate TLR9 (Yasuda et al. 2005). The high mobility group box chromosomal protein 1 (HMGB1), produced by both spermatogonia and Sertoli cells, is a TLR2 and 4 ligand (Yu et al. 2006; Zetterström et al. 2006). Expression of TLRs by the Sertoli cells under normal conditions, therefore, suggests a potential role for these receptors in responding to these endogenous ligands.

11.10.4.2.2 The interleukin 1 family

IL1 is the best studied of all the cytokines normally expressed in the testis. It is produced in two forms, α and β, which are encoded by separate genes and share approximately 25% sequence homology. Both forms act through the same receptor complex (IL1R1), and exert a similar range of proinflammatory and physiological effects (Dinarello 1996; O'Neill and Dinarello 2000). Most IL1 signaling occurs through activation of the MyD88/TRAF and Jnk/p38 MAP kinase pathways, thereby regulating many proinflammatory genes through stimulation of the transcription factors NFkB and activated protein 1 (AP1) (Medzhitov et al. 1998). The IL1s are synthesized as precursor proteins, which are cleaved to produce 17 kDa active proteins. The precursor of IL1α, the precursor possesses low-level biological activity, but the IL1β precursor protein is inactive (Black et al. 1988; Watanabe and Kobayashi 1994). The precursor of IL1β is cleaved by the IL1 converting enzyme (ICE, caspase 1) during the pro-form during inflammation, whereas IL1α is more commonly found within the cell, where it may act as an autocrine or paracrine growth factor involved in direct cell-to-cell communication.

In addition to the prototypical IL1α and IL1β, the IL1 family comprises a number of structurally related proteins, which appear to have arisen by gene duplication (Dunn et al. 2001). The closest structurally to IL1α/β is IL18, which is processed by caspase 1 activity, but acts via a separate receptor rather than IL1R1 (Gracie et al. 2003). Another member of the family, which does bind to IL1R1 but lacks the ability to transduce a signal, blocks IL1α/β action and is called IL1 receptor antagonist (IL1ra) (Arend 1991).

Production of IL1α is constitutively high in the normal testis, as IL1α is produced by the Sertoli cells in a cyclical manner within the mature seminiferous epithelium (Jonsson et al. 1999; Söder et al. 1991). Its production is driven by the developing germ cells and by phagocytosis of the residual cytoplasm cast off by the spermatids at the time of their release into the tubule lumen (Gérard et al. 1992; Syed et al. 1995). Sertoli cell IL1α production is also stimulated by LPS, but not by FSH (Stéphan et al. 1997). Production of IL1β by the normal testis is low, but is upregulated in a subset of testicular macrophages and the Leydig cells during inflammation (Gérard et al. 1991; Jonsson et al. 1999; O'Bryan et al. 2005). Curiously, the majority of this secreted testicular IL1β exists as the precursor (O'Bryan et al. 2005).

The receptor IL1R1 has been localized to germ cells and Sertoli cells (Gomez et al. 1997). IL1α stimulates DNA synthesis in spermatogonia (i.e., mitosis) and early spermatocytes (i.e., meiosis), proliferation of immature Sertoli cells, and a number of activities of the mature Sertoli cell that support spermatogenesis, such as the production of lactate and transferrin (Hakovirta et al. 1993b; Hoeben et al. 1996; Nehar et al. 1998; Parvinen et al. 1991; Pöllänen et al. 1989; Söder et al. 1991). It also regulates the ability of the Sertoli cell to maintain contact with other Sertoli cells and the developing germ cells, by altering the Sertoli cell cytoskeleton (Sarkar et al. 2008). In the interstitium, both IL1α and IL1β suppress LH-stimulated androgen production by adult Leydig cells through inhibition of steroidogenic enzyme activity (Hales 1992; Xiong and Hales 1994, 1997). IL1ra is produced by the Sertoli cell, and is stimulated by FSH, IL1, and LPS (Zeyse et al. 2000). Presumably, its role is to regulate the activity of IL1α/β within the testis.

Both IL18 and its receptor have been found in the seminiferous epithelium, with IL18 mRNA and protein localized to spermatocytes and round spermatids (Strand et al. 2005). As is the case for IL1β, the majority of IL18 produced within the testis is in the precursor form, indicating that the processing of these cytokines by caspase 1 in the testis is an area requiring more investigation. IL18 stimulates spermatogonial DNA synthesis in cultures of rat seminiferous tubules, without influencing germ cell apoptosis (Strand et al. 2005). Another member of the family localized to the testis is IL1F8, although its function there is entirely unknown (Kumar et al. 2000).


11.10.4.2.3 Tumor necrosis factor α and Fas ligand

TNFα and FasL are transmembrane and secreted cytokines with typically trimeric structures and they interact with specific receptors to mediate a cell death signal (Ju et al. 1995; Schütze et al. 2008). They are implicated in both immunoregulation and the control of normal spermatogenic function in the testis.

TNFα is a 17 kDa glycosylated polypeptide and is a major early product of activated monocytes and macrophages. It binds to either of two TNF receptor subsets (TNFR1 and TNFR2) and plays a central role in initiating the inflammatory response by stimulating the production of IL1 and IL6 (Basak and Hoffmann 2008; Bradley 2008). Whether TNFα exerts proinflammatory or cytotoxic effects depends on the receptor subtype engaged and the expression of specific adaptor proteins within the target cell (Basak and Hoffmann 2008; Chung et al. 2002; Hsu et al. 1995; Mak and Yeh 2002). Consistent with its name, TNFα exerts a cell death signal via TNFR1, through interaction with the TNFR-associated death domain protein (TRADD) or the Fas-associated death domain protein (FADD), and activation of the caspase-dependent apoptotic pathway (Schütze et al. 2008). However, interaction with TRADD can also result in recruitment of other adaptor proteins leading to the binding of TRAF2 and activation of the NFκB pathway and/or the MAP kinases Jnk or p38 (Chung et al. 2002). Moreover, TNFR2 and its related receptors, which include CD40, do not contain a death domain, and instead associate with the TRAFs leading to activation of cell signaling events (Bradley 2008; Mak and Yeh 2002).

In the mouse testis, TNFα is expressed by round spermatids, pachytene spermatocytes, and testicular macrophages, and mRNA for TNFR1 has been located on both Sertoli and Leydig cells (De et al. 1993). In porcine Sertoli cells, TNFα receptor subunit protein expression is stimulated by FSH (Mauduit et al. 1996). There is no evidence that TNFα is produced by the Sertoli cell, but expression of TNFα by the germ cells within the seminiferous epithelium, like that of IL1α and IL6, is cyclical. Within the seminiferous epithelium, TNFα produced by the germ cells appears to play a complex role in the control of both Sertoli cell function and spermatogenesis. TNFα reduces spontaneous germ cell degeneration in cultured human and rat seminiferous tubules, suggesting a germ cell survival effect presumably mediated through the Sertoli cell (Pentikäinen et al. 2001; Suominen et al. 2004). Conversely, TNFα disrupts Sertoli cell tight junction assembly by inhibiting the production of junction proteins and by regulating matrix metalloprotease and protease inhibitor activity (Siu et al. 2003). TNFα also stimulates plasminogen activator inhibitor expression in rat testicular peritubular cells (Le Magueresse-Battistoni et al. 1997). Similar to IL1, TNFα stimulates basal lactate production by cultured Sertoli cells, but TNFα generally antagonizes the actions of FSH on Sertoli cell function, including the stimulation of aromatase activity and lactate production (Mauduit et al. 1993; Nehar et al. 1997; Riera et al. 2001). However, Delfino et al. (2003) have shown that TNFα stimulates androgen receptor expression in Sertoli cells via upregulation of NFκB, which binds to several enhancer motifs in the androgen receptor promoter. TNFα also stimulates the expression of inflammatory cytokines, chemokines, and leukocyte adhesion molecules in both Sertoli cells and peritubular cells (Aubry et al. 2000; De Cesari et al. 1998; Stéphan et al. 1997). In testicular pathology, TNFα has been implicated as a major causative agent in the development of experimental autoimmune orchitis (EAO) (Yule and Tung 1993). There is a significant increase in the number of TNFα-positive testicular macrophages and the number of TNFR1-positive germ cells undergoing apoptosis during EAO in rats (Suescun et al. 2003). Within the interstitium, TNFα acts as an effective regulator of Leydig cell steroidogenesis, by inhibiting LH receptor binding and steroidogenic gene expression (Li et al. 1995; Mauduit et al. 1991b, 1998; Xiong and Hales 1993b, 1994).

No intratesticular cytokine has excited more controversy than the cell death signaling ligand FasL. Interactions between FasL and its receptor on activated T cells are important in the regulation of the immune response (Ju et al. 1995). The death domain in the cytoplasmic region of the Fas receptor recruits the FADD adaptor protein and induces T cell death via caspase-dependent apoptosis (Schütze et al. 2008). There is evidence that FasL expression on epithelial cells in immune-privileged tissues, including Sertoli cells, may be responsible for deleting activated T cells within the tissue, thereby suppressing adaptive immunity (Bellgrau et al. 1995; Griffith et al. 1996; Saas et al. 1997; Stuart et al. 1997). However, this hypothesis has been challenged by the observation that increased FasL expression on cells such as tumor cell lines and pancreatic islet cells does not increase protection in transplantation studies, but actually
provokes a massive inflammatory reaction and rejection (Allison et al. 1997; Kang et al. 1997). Moreover, FasL appears to be abundantly expressed in the epithelia of several human tissues that lack any evidence for immunological privilege (Xerri et al. 1997), while absence or inhibition of Fas or FasL does not automatically cause failure of immune privilege (Rogers et al. 1998; Suarez-Pinzon et al. 2000; Wahlsten et al. 2000). It seems unlikely that FasL expression on its own is a fundamental explanation for immune privilege, although it may contribute through interaction with other immunoregulatory processes (Chen et al. 1998).

Localization of FasL and Fas in the testis under normal conditions also has proven controversial, which may be attributed to differences in detection methods, limitations of some of the reagents that have been used, and the fact that these molecules are inducible (D’Alessio et al. 2001; Restifo 2000). Studies have shown FasL to be present in rat, mouse, porcine, and human Sertoli cells and absent in most germ cells (Bellgrau et al. 1995; Brændstrup et al. 1999; D’Abrizio et al. 2004; Lee et al. 1997), but other studies have reported that FasL expression in the rat seminiferous epithelium is confined to the germ cells (Celik-Ozenci et al. 2006; D’Alessio et al. 2001). The receptor Fas has been found on isolated mouse Sertoli cells (Riccioli et al. 2000), but in intact testes has been localized to spermatogonia and spermatocytes from the pubertal period onward (Celik-Ozenci et al. 2006; Lee et al. 1997; Lizama et al. 2007; Ogí et al. 1998). In germ cells, Fas expression is associated with cells that are undergoing apoptosis (Lee et al. 1997; Lizama et al. 2007), and Fas is induced by TNFα and IFNγ in the Sertoli cell (Riccioli et al. 2000). Curiously, in one study using fragment cultures of human seminiferous tubules, TNFα downregulated FasL expression and inhibited apoptosis of germ cells (Pentikäinen et al. 2001). Less controversially, Fas and FasL expression is upregulated in various models of seminiferous epithelium damage, indicating that this mechanism is important in regulating germ cell apoptosis in cases of physical and toxicological insult (Lee et al. 1997; Ogí et al. 1998).

11.10.4.2.4 Interleukin 6 and the gp130 family

Members of the IL6 family of cytokines exert their actions via binding to specific receptors that associate with a common membrane signal transducer gp130, leading to the activation of the Janus kinase/signal transducers and activators of transcription (Jak/Stat) and MAP kinase cascades (Heinrich et al. 2003). In addition to their functions in inflammation and the immune response, these cytokines play crucial roles in hematopoiesis, liver and neuronal regeneration, embryonic development, and fertility. Members of this family that have been detected in the testis are leukemia inhibitory factor, oncostatin M, ciliary neurotropic factor, IL11, and IL6 itself (de Miguel et al. 1996, 1997; Du et al. 1996; Jenab and Morris 1998; Okuda et al. 1994; Stéphan et al. 1997). The principal roles of most of these cytokines in the testis appear to lie in regulating Leydig cell development and the onset of spermatogenesis. A role in testicular immunology is most likely for IL6, which possesses both proinflammatory and anti-inflammatory properties, and regulates the acute-phase response as well as several aspects of immune cell development and activity (Kopf et al. 1994; Tilg et al. 1997).

Sertoli cells, Leydig cells, and peritubular cells have been shown to produce IL6 in vitro (Cudicini et al. 1997b; Okuda et al. 1994; Syed et al. 1993, 1995). In rat Sertoli cells, IL6 production is stimulated by FSH, testosterone, phagocytosis, and other inflammatory stimuli, including IL1α, IL1β, TNFα, and LPS (Cudicini et al. 1997a,b; Okuda et al. 1994, 1995; Stéphan et al. 1997; Syed et al. 1995). Production of IL6 by cultured mouse Sertoli cells is inhibited by INFγ (Riccioli et al. 2000; Stéphan et al. 1997). Within the seminiferous epithelium, endogenous production of IL6 follows a cyclical pattern that corresponds with the changes in the stages of the spermatogenic cycle, most probably under the influence of IL1α and FSH (Hakovirta et al. 1995; Syed et al. 1993, 1995). Both the IL6-specific receptor subunit (IL6R) and the gp130 mRNA are expressed in rat Sertoli cells, and are stimulated by IL1 and IL6, but only the IL6R subunit is stimulated by FSH (Fujisawa et al. 2002). IL6 increases basal and FSH-induced transferrin and cyclic GMP secretion by the Sertoli cell, and inhibits meiotic DNA synthesis in preleptotene spermatocytes (Boockfor and Schwarz 1991; Hakovirta et al. 1995; Hoeben et al. 1997). In models of EAO, IL6 appears to play an ameliorative or protective role within the seminiferous epithelium (Li et al. 2002; Rival et al. 2006b). Leydig cells are also active producers of IL6 following stimulation by LH, LPS, or IL1β in vitro, and evidence suggests that these cells actually may be the major testicular source (Boockfor et al. 1994; Cudicini et al. 1997b; Okuda et al. 1994, 1995).

11.10.4.2.5 Interleukin 10

Several studies have implicated IL10 as a key player in testicular immunology. Testicular IL10 production is preferentially induced by systemic LPS treatment in
the adult rat testis (O'Bryan et al. 2005), and macrophages and T cells isolated from the rat testis constitutively produce IL10, which may contribute to testicular immune privilege (Hedger, unpublished data). In a mouse model of EAO, elevation of endogenous IL10 levels by adenoviral-mediated transfection of human IL10 was found to significantly reduce the incidence of immune activation, orchitis, and spermatogenic damage (Watanabe et al. 2005). Although male mice deficient in IL10 do not show evidence of male fertility problems, intratesticular immune responses have yet to be examined in these animals (Kühn et al. 1993).

11.10.4.2.6 Nitric oxide and oxygen metabolites

Production of ROS, particularly by macrophages, is an important component of the early response to infection (Takemura and Werb 1984). Significant ROS include the superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), the hydroxyl radical (HO$^\cdot$), nitric oxide (NO$^\cdot$), and the peroxynitrite anion (ONOO$^-$). Most are products of normal cellular metabolism, but their production is stimulated during inflammation through the activity of the enzymes NADPH oxidase (nicotinamide adenine dinucleotide phosphate-oxidase) and iNOS (Forman and Torres 2002; Nathan 2006). These small molecules are cytotoxic to many microorganisms, although some play a more complex role in cell signaling processes. They also interact with crucial intracellular macromolecules, such as proteins, lipids, and DNA, causing oxidative damage. Although there are endogenous repair systems to correct oxidative damage, excessive production of ROS contributes to the pathology of many diseases.

Among the ROS, nitric oxide represents a special case. At high levels, NO is highly cytotoxic, especially as a precursor of the peroxynitrite anion, but at low levels NO acts as an intracellular and extracellular signaling molecule (Bogdan 2001; Schmidt and Walter 1994). It is produced by one of three related enzymes, neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3), which catalyze the conversion of L-arginine to L-citrulline and NO (Alderton et al. 2001). The NOS enzymes are homodimeric proteins encoded by three separate genes (Table 2). Both nNOS and eNOS are constitutively expressed enzymes whose activity is regulated through a calcium–calmodulin-mediated mechanism, whereas iNOS is a constitutively activated enzyme that is regulated at the transcriptional and translational levels by various inflammatory mediators, including LPS, IL1, and TNF$\alpha$ (Wolkow 1998).

In studies in various species, NOS has been found in Sertoli cells, Leydig cells, peritubular cells, germ cells, testicular macrophages, and vascular endothelial cells (Kim et al. 2007; Lee and Cheng 2004). The nNOS gene also produces a testis-specific isoform, TnNOS, which has been localized to Leydig cells (Wang et al. 1997). In the normal rat seminiferous epithelium, iNOS is expressed by elongating spermatids and pachytene spermatocytes, particularly during the stages immediately following sperm release, with relatively lower levels of expression in Sertoli cells and peritubular cells throughout the

**Table 2** Major inflammatory enzymes – properties and distribution in the testis

| Enzyme                              | Properties                                      | Principal cellular expression in normal testis                        |
|-------------------------------------|-------------------------------------------------|-----------------------------------------------------------------------|
| Neuronal nitric oxide synthase (nNOS, type I) | Ca/calmodulin regulated, constitutively expressed | Sertoli cell, Leydig cell, vascular endothelium                        |
| Inducible nitric oxide synthase (iNOS, type II) | Constitutively active, transcriptionally and translationally regulated | Sertoli cell, Leydig cell, peritubular cell, pachytene spermatocytes and elongating spermatids, macrophage subset |
| Endothelial nitric oxide synthase (eNOS, type III) | Ca/calmodulin regulated, constitutively expressed | Sertoli cell, Leydig cell, peritubular cell, vascular endothelium     |
| Phospholipase A$_2$                 | Multiple classes and groups, various properties  | Ubiquitous expression                                                 |
| Cyclooxygenase 1 (COX1)             | Constitutively expressed, not induced by inflammation | Ubiquitous expression                                                |
| Cyclooxygenase 2 (COX2)             | Induced by inflammation                         | Sertoli cell, Leydig cell, peritubular cell, all spermatogenic cells  |
entire cycle (O’Bryan et al. 2000a). In the interstitium, macrophage expression of iNOS appears to be largely confined to the minority of CD163-negative macrophages of the testis, and is not detectable in the majority of resident macrophages even during inflammation (O’Bryan et al. 2000a; Gerdprasert et al. 2002a). Testicular cell iNOS expression and NO production are increased by inflammatory events induced by LPS, testicular torsion, or testicular heating (Lue et al. 2003; O’Bryan et al. 2000a; Shiraishi et al. 2001).

Production of NO by germ cells, in particular, is implicated in the control of the formation and disassembly of the Sertoli cell junctions that constitute the blood–testis barrier, as well as the junctional complexes involved in Sertoli–germ cell adhesion (Lee and Cheng 2003; Lee et al. 2005). Moreover, pachytene and round spermatid apoptosis is significantly reduced in iNOS null mice, leading to an increase in daily sperm output and indicating a key role for iNOS in limiting germ cell survival and/or the carrying capacity of the Sertoli cells (Lue et al. 2003). NO inhibits Leydig cell steroidogenesis directly, an action that is probably mediated through oxidative damage, and treatment with NOS inhibitors counteracts the normal decrease in testosterone associated with sepsis or stress (Del Panta et al. 1996; Sharma et al. 1998; Welch et al. 1995). Similarly, production of other ROS has been implicated in the loss of steroidogenic function in various inflammatory models and in Leydig cell cultures (Georgiou et al. 1987; Quinn and Payne 1984). Last but not least, NO is a potent vasodilator, and plays a role in the endogenous control of testicular blood flow and formation of the interstitial fluid (Lissbrant et al. 1997; O’Bryan et al. 2000a). Production of NO is an important mediator of germ cell death in testicular torsion (Moon et al. 2005; Shiraishi et al. 2001), and may be involved in the testicular damage associated with varicocele (Santoro et al. 2001; Türker Köksal et al. 2004).

11.10.4.3 Inflammatory Signaling Pathways in the Seminiferous Epithelium

It is evident that the major inflammatory signaling pathways mediated via NFκB and the p38/Jnk MAP kinases play important, albeit complex, intratesticular roles. Notably, NFκB has been implicated as an apoptosis-inducing signal for germ cells in numerous studies (Lysiak et al. 2005; Pentikäinen et al. 2002; Rasoulpour and Boekelheide 2005; Starace et al. 2005), even though it stimulates androgen receptor expression in the Sertoli cell (Delfino et al. 2003). Activation of p38/Jnk is implicated in the stimulation of proliferation by immature Sertoli cells and the regulation of blood–testis barrier dynamics, steroidogenesis, and multiple inflammatory responses, including the production of IL6, iNOS, monocyte chemoattractant protein 1, and leukocyte adhesion molecules, in the mature Sertoli cell (De Cesaris et al. 1998, 1999; Ishikawa and Morris 2006; Ishikawa et al. 2005; Petersen et al. 2005; Riccioli et al. 2006). While these pathways mediate many of the effects of inflammation on the testis, endogenous regulation of these pathways also appears to be involved in the normal functions of the seminiferous epithelium, as will be discussed later.

11.10.4.3.1 Interferons

The IFNs are functionally related cytokines with antiviral activity and they comprise three main groups (α, β, and γ), based on their structural relationships and major cellular sources (Hertzog et al. 2003; Malmgaard 2004). The type I IFNs (IFNα and β) are produced by a variety of cell types in addition to leukocytes, and exert primarily antiviral and anti-proliferative effects via the IFNα receptor (IFNAR). The type II IFNγ is produced by NK and NK T cells, activated T cells and, under certain conditions, by macrophages and dendritic cells (Schoenborn and Wilson 2007; Varma et al. 2002). It acts through a separate receptor, and regulates the activity of APCs as part of the adaptive immune response in addition to its antiviral functions. Hence, IFNγ is a type II IFN, but a type 1 cytokine.

Type I IFN production is stimulated through TLR3, which detects double-stranded viral RNA, and TLR4, the bacterial LPS receptor (Hertzog et al. 2003). These two TLRs, which are expressed in the testis (Bhushan et al. 2008; Riccioli et al. 2006; Starace et al. 2008; Wu et al. 2008), are able to induce the IFN transcription factor IRF3, via engagement of the TRIF adapter protein (Akira and Takeda 2004; Hertzog et al. 2003; O’Neill and Bowie 2007). The regulation of IFNγ is cell specific and complex, but involves Jak/Stat signaling and various transcription factors, including NFκB and AP1 (Schoenborn and Wilson 2007; Varma et al. 2002). Its production is stimulated by type I IFNs and by IL18, among other cytokines. Viral infections stimulate type I IFN and, somewhat more surprisingly, IFNγ production by Sertoli cells, peritubular cells, Leydig cells, and testicular macrophages, leading to typical antiviral responses in the testis (Dejucq et al. 1995,
1998a,b; Hu et al. 1998; Starace et al. 2008). A role for IFNγ in maintaining testicular immune privilege has been indicated by the observation that mouse Sertoli cells upregulate their expression of the negative costimulatory ligand B7-H1 in response to IFNγ in vitro, but remain devoid of positive costimulatory molecules (Dal Secco et al. 2008). On the other hand, in vivo studies have identified IFNγ as a causative factor in EAO in mice (Itoh et al. 1998b). Moreover, IFNγ stimulates Sertoli cell production of Fas and caspase 1, and is implicated as the mediator of Sertoli cell and germ cell apoptosis under various conditions (Kanzaki and Morris 1998; Riccioli et al. 2000). These disparate observations are indicative of a complex relationship between IFNγ, the local immune system, and spermatogenesis.

IFNα production also affects testicular steroidogenesis. Normal healthy men treated with human IFNα had significantly decreased serum testosterone levels in conjunction with normal serum gonadotropins, and both IFNα and IFNγ inhibit testosterone production in primary cultures of Leydig cells (Orava et al. 1986). Studies using porcine Leydig cells indicate that IFNγ exerts its inhibitory effect on testosterone production at the level of cholesterol transport into the mitochondria, and inhibits expression of the steroidalogen acute regulatory (StAR) protein and the P-450 steroidalogen enzymes, cholesterol side-chain cleavage complex (P450scC) and 17α-hydroxylase/C17–C20 lyase (P450c17) (Orava et al. 1985, 1989). These data indicate that IFNs may contribute to the decline in steroidalogen function of patients with viral infections, in particular. However, Dejucq et al. (1995, 1998a) have shown that an increase in IFNα and IFNγ expression in Sertoli and Leydig cells of rats infected with Sendai virus was actually associated with an increase in testosterone production. These results suggest that indirect or secondary stimulatory effects on testicular steroidogenesis may be involved, providing further evidence that IFNs play a complex role in testicular function.

### 11.10.4.3.2 Transforming Growth Factor β and Activin A

The three TGFβ family members that are expressed in mammals (TGFβ1, TGFβ2, and TGFβ3) are the archetypes of a much larger superfamily of mostly homo- and heterodimeric proteins, which includes the activins, the bone morphogenetic proteins (BMPs), inhibin, and anti-Müllerian hormone (Lin et al. 2006). The TGFβs themselves are multifunctional growth and differentiation factors involved in many aspects of tissue remodeling and repair as well as regulation of the immune system (Ashcroft 1999; Chang et al. 2001; Licona-Limón and Soldevila 2007). Nearly all cells synthesize a form of TGFβ and possess functional receptors for these cytokines. Most TGFβ family ligands act via dual transmembrane serine/threonine kinase receptors, called type I and type II, which interact upon ligand binding (Cárcamo et al. 1994; Ebner et al. 1993; Massagué et al. 1992). Signals are transduced from the membrane to the nucleus through phosphorylation of the regulatory Smad signaling proteins, with Smads 2, 3, and 4 responsible for mediating TGFβ and activin signaling (Datto et al. 1999; de Caestecker et al. 1998).

All three mammalian TGFβ forms are differentially expressed by Sertoli cells, peritubular cells, and Leydig cells in the fetal and immature testis, but production declines considerably during sexual maturation (Avallat et al. 1994; Gautier et al. 1994; Mullaney and Skinner 1993; Olaso et al. 1997). In the postpubertal testis, they have also been localized to the germ cells (Caussanel et al. 1997; Teerds and Dorrington 1993), and TGFβ receptors are found in both somatic and germ cells (Caussanel et al. 1997; Goddard et al. 2000; Le Magueresse-Battistoni et al. 1995). The TGFβs appear to be involved in testicular development by controlling, among other things, apoptosis of undifferentiated spermatogonia (gonocytes) (Olaso et al. 1998), seminiferous tubule formation, and Leydig cell differentiation (Cupp et al. 1999; Dickson et al. 2002; Khan et al. 1992a; Konrad et al. 2000). In the adult testis, TGFβ2 and TGFβ3 regulate Sertoli cell tight junction dynamics, indicating a role in regulating the permeability of the blood–testis barrier to facilitate the passage of spermatocytes across the barrier during spermatogenesis (Lui et al. 2003; Xia et al. 2006). However, the TGFβs are also potent inhibitors of the activity of immune cells, especially T cells, B cells, and NK cells (Ahmad et al. 1997; Ashcroft 1999; Cousins et al. 1991; Licona-Limón and Soldevila 2007; Wahl et al. 2006), suggesting a prominent role in creating testicular immune privilege. Accordingly, TGFβ1 contributes to the lymphocyte inhibitory activity of testis extracts (Pollänen et al. 1993) and to the immunoprotective properties of Sertoli cells in cotransplantation studies (Suarez-Pinzon et al. 2000).

Activins are homodimers or heterodimers of homologous subunits, designated βA−βE, which are structurally related to the TGFβs (Chang et al. 2001; de Kretser et al. 2002). Homodimers of βA form activin A, which is widely expressed and has been extensively studied. Relatively less is known about
the other activin forms, which appear to be both less abundant and less widely distributed. Activin A is a multifunctional growth factor and inflammatory regulator (Phillips et al. 2001), although activins were originally named for the ability of activin A and B, in particular, to stimulate pituitary FSH secretion (Cameron et al. 1991; Ling et al. 1986). Heterodimers of either $\beta_A$ or $\beta_B$ subunits with a homologous $\alpha$ subunit, are called inhibin A and inhibin B, respectively, and act as feedback inhibitors of FSH secretion from the pituitary (Robertson et al. 1986). Unlike the activins, inhibins are not widely produced, and their chief source is the Sertoli cell, the cellular target of FSH action (de Kretser et al. 2004; Meunier et al. 1988).

Activin functions are highly regulated. Like the TGF$\beta$s, they act via specific type I and type II transmembrane serine/threonine kinase receptors, linked to the Smad signaling pathway (Ethier and Findlay 2001; Vale et al. 2004). The inhibins are competitive inhibitors of activin receptor binding, but activin homodimers and heterodimers comprising the $\beta_B$ and $\beta_C$ subunits also appear to act as weak competitive agonists of activin A (Brown et al. 2000; Makanji et al. 2008; Mellor et al. 2003). These interactions are modulated and/or facilitated by specific inhibitory coreceptor proteins, such as betaglycan and the BMP and activin membrane-bound inhibitor (BAMBI) (Lewis et al. 2000; Makanji et al. 2008; Onichtchouk et al. 1999). Moreover, activin bioactivity can be effectively neutralized in the circulation by the high-affinity activin binding protein, follistatin (Nakamura et al. 1991).

Activin A is abundantly produced in the testis, particularly during early testicular development (Barakat et al. 2008; Buzzard et al. 2004). Even in the adult rat, intratesticular fluid levels of activin A are 5–10 times higher than normal circulating concentrations (O’Bryan et al. 2005). The Sertoli cells appear to be the main source in the adult testis, although the $\beta_A$ subunit is expressed in most germ cells (Buzzard et al. 2004; Kaipia et al. 1992), and activin A protein is present in the resident macrophages and mast cells (Okuma et al. 2005b, 2006). Activin A is expressed at low levels throughout the cycle of the seminiferous epithelium, but there is a distinct peak of production immediately following spermiation, which is driven by the surge of IL1$\alpha$ produced by the Sertoli cell at this time (Kaipia et al. 1992; Okuma et al. 2006). Activin receptors are expressed by most, if not all, of the somatic and germ cells in the testis (Cameron et al. 1994; de Winter et al. 1992; Feng et al. 1993; Kaipia et al. 1993). Activin A exerts a complex regulation of germ cell, Sertoli cell, and Leydig cell proliferation and/or differentiation during development and in the adult (Barakat et al. 2008; Buzzard et al. 2003; Mather et al. 1990; Mauduit et al. 1991a; Meehan et al. 2000; Meinhardt et al. 2000), and disorders of activin signaling are implicated in the onset of testicular cancer (Dias et al. 2008).

Activin A is produced by activated monocytes, macrophages, dendritic cells, bone marrow stromal cells, and some lymphocytes (Erämaa et al. 1992; Ogawa et al. 2006; Robson et al. 2008; Yamashita et al. 1992), and plays a facilitating role in early inflammation responses (Jones et al. 2007). Consistent with its homology to the TGF$\beta$s proteins, however, activin A also possesses a repertoire of anti-inflammatory and immunoregulatory activities, and displays characteristics of a type 2 cytokine (Hedger et al. 1989; Ogawa et al. 2006; Wang et al. 2008; Yu et al. 1998). In various experimental systems, activin A has been found to antagonize the production and actions of IL1 and IL6, to inhibit critical T cell and B cell activation responses, and to induce macrophages to polarize toward the M2 phenotype (Brosh et al. 1995; Gribi et al. 2001; Ogawa et al. 2006; Russell et al. 1999). Both Sertoli cells and testicular macrophages in culture respond to LPS by increasing activin A production (Okuma et al. 2005a) (Hedger, unpublished data), and IL1 is a very potent stimulus for activin A production by the Sertoli cell (Okuma et al. 2005b, 2006). The actual role of activin A in testicular inflammation remains unclear, since the high intratesticular levels of activin A are not affected by LPS treatment in vivo (O’Bryan et al. 2005), but a role for activin A in modulating testicular immune responses can be predicted.

11.10.4.3.3 Steroids

Androgens produced by the Leydig cells have immunosuppressive properties that contribute to differences in immunity between the sexes (Cutolo et al. 2004; Miller and Hunt 1996). The majority of these effects are believed to be exerted at the level of the immune tissues, rather than by direct effects on circulating leukocytes, which lack classical androgen receptors (Grossman et al. 1979; Sasson and Mayer 1981). Recently, however, it has been discovered that steroids can interact with membrane-bound G-protein-coupled receptors to trigger nongenomic responses (Braun and Thomas 2004; Rahman and Christian 2007). Studies have shown that androgens alter [Ca$^2+$] fluxes in lymphocytes and macrophages via such membrane-mediated interactions, affecting
gene expression and function in the target cells (Benten et al. 2004; Walker 2003; Wunderlich et al. 2002). Although studies of the role of androgens in suppressing immune responses, such as graft rejection, in the testis have been somewhat equivocal (Cameron et al. 1990; Selawry and Whittington 1988; Whitmore and Gittes 1978), the observation that immune cells can respond directly to androgens has resurrected the intriguing possibility that androgens, and possibly other testicular steroids, may directly regulate immune cell activity within the testis and adjacent draining lymph nodes.

Glucocorticoids play an important role in modulating both testicular immunity and steroidogenesis via a complex extratesticular regulatory loop. During inflammation, proinflammatory cytokines activate the hypothalamic–pituitary–adrenal axis leading to increased secretion of glucocorticoids, which act through the ubiquitously expressed glucocorticoid receptors (Buckingham et al. 1996; Sapolsky et al. 2000). Glucocorticoids suppress innate and acquired immunity at multiple levels and are an essential control in the inflammatory/immune response. They inhibit the inflammatory response primarily by repression of NFκB, suppressing the production and actions of the proinflammatory cytokines, reducing adhesion molecule expression, and stimulating the production of anti-inflammatory cytokines (Auphan et al. 1995; Brack et al. 1997; Kapcala et al. 1995). Glucocorticoids also exert inhibitory effects at all levels of the hypothalamic–pituitary–Leydig cell axis (Bambino and Hsueh 1981; Hales and Payne 1989; Hardy et al. 2005; Monder et al. 1994), thereby contributing to the inhibition of androgen production that accompanies local and systemic inflammatory events.

### 11.10.4.3.4 Bioactive lipids: Prostanoids and phospholipids

Prostaglandins (PGD, PGE, and PGF), prostacyclins (PGI), and thromboxanes (Tx), collectively called the prostanoids, are fundamental to many physiological processes, including inflammation and immunity. Prostanoids arise from hydrolysis of membrane glycerophospholipids to release the free fatty acid arachidonic acid through the action of one of the more than 20 enzymes with phospholipase A2 (PLA2) activity (Kudo and Murakami 2002; Schaloske and Dennis 2006). Arachidonic acid is in turn directed down the prostanoid synthetic pathway by the action of the cyclooxygenase (COX; also called prostaglandin-endoperoxide synthase) enzymes (Figure 3).

There are two COX enzymes, which are the products of different genes: the constitutively expressed COX1 and an inflammation-induced form, COX2 (Table 2) (Smith et al. 2000; Tanabe and Tohnai 2002). While conversion of arachidonic acid to the prostanoid precursor PGH2 by COX is the rate-limiting step, production of specific prostanoids depends on the activity of the prostaglandin synthases, that is, PGE synthase (PGES), PGIS, PGDS, PGFS, and thromboxane A synthase (TxAS) (Helliwell et al. 2004; Ueno et al. 2005). Each prostanoid acts via one or more specific receptors to regulate cell growth, vascular smooth muscle constriction or relaxation, vascular permeability, and immune cell activity (Bos et al. 2004; Hata et al. 1990). Several prostanoids exert both proinflammatory and immunosuppressive actions through different receptor interactions, or through metabolism to other active immunoregulatory metabolites, such as 15-deoxy-PGJ2, a ligand for the peroxisome proliferator-activated receptor γ (PPARγ) (Jiang et al. 1998). An alternative synthetic pathway, catalyzed by the lipoxygenases, converts arachidonic acid to the related leukotrienes and lipoxins, which also possess diverse metabolic, vascular, and inflammation-regulating actions (Samuelsson et al. 1987; Takano et al. 1997).

Most of the enzymes responsible for prostanoid production are ubiquitously expressed, and this includes the male reproductive tract (Gerena et al. 2000; Lazarus et al. 2002; Takahashi et al. 1995; Winnall et al. 2007). In fact, the levels of E series prostaglandins in human seminal plasma are remarkably high (Cooper and Kelly 1975). Most testicular cells express both COX forms, and possess the capacity to produce prostaglandins in vitro (Winnall et al. 2007), although there appear to be cell type-specific and species-specific differences in the relative levels of expression (Balaji et al. 2007; Frungieri et al. 2006; Lazarus et al. 2004). In the normal rat testis, COX2 is responsible for the majority of prostaglandin production (Winnall et al. 2007), although intratesticular PGE2 levels are only marginally affected by acute inflammation (Winnall et al. 2008). This is probably due to the fact that macrophages in the rat testis express COX2 at very low levels, but are the only testis cell type to respond to an inflammatory stimulus with a significant increase in COX2 activity (Winnall et al. 2007). These data point toward a previously unanticipated maintenance role for the so-called inducible COX2 enzyme in testicular function, as well as an anomalous inflammatory response of testicular COX2, consistent with an altered capacity
Fertility is retained in male mice lacking either COX1 or COX2, whereas double deletions are lethal (Langenbach et al. 1999; Lim et al. 1997; Sales and Jabbour 2003). Studies on male fertility and spermatogenesis using prostaglandins, or nonsteroidal anti-inflammatory drugs such as aspirin and indomethacin, which act primarily via inhibition of COX activity, have produced conflicting conclusions (Abbatio et al. 1975; Biswas et al. 1978; Sanyal et al. 1980; Winnall et al. 2008). These diverse outcomes indicate the complexity of the roles of prostanoids in controlling various aspects of testicular function. Prostaglandins, particularly PGE2 and PGF2α, have been implicated in the control of Leydig cell development in the immature testis, production of proinflammatory cytokines by the Leydig and Sertoli cells, autoregulation of steroidogenesis in the adult, and the decline in Leydig cell function that occurs during aging (Baker and O'Shaughnessy 2001; Cooke et al. 1991; Haour et al. 1979; Ishikawa et al. 2005; Walch and Morris 2002; Wang et al. 2005). Production of PGE2 and PGF2α by COX2 also mediates the effects of IL1 on protein and lipid regulation by the Sertoli cell involved in supporting inflammatory reactions.

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Figure 3 Prostanoid and lysophosphatidylcholine synthesis and targets. Synthesis of the prostanoids commences through the action of phospholipase A2 (PLA2) on arachidonyl-containing phospholipids (usually a 1-acyl-2-arachidonyl-glycerophospholipid) to produce the free fatty acid arachidonic acid and the corresponding lysophospholipid. Arachidonic acid is converted into the prostanoid precursor prostaglandin H2 (PGH2) by the action of cyclooxygenases and this is further converted into the various prostanoid subtypes, prostaglandins (PGD, PGE, and PGF), prostacyclins (PGI), and thromboxanes (Tx), by the action of specific prostaglandin and thromboxane synthases. Synthesis of lipoxins and leukotrienes from arachidonic acid occurs via the activity of a separate lipoxigenase enzyme (not shown). The prostanoids interact with their various receptor subtypes: there are two PGD receptors (DP1–2) and four PGE receptors (EP1–4). It is the cellular expression of these receptor subtypes that ultimately determines the eventual biological responses; for example, different prostanoid and receptor combinations may produce either proinflammatory or anti-inflammatory responses, or may have opposing vasodilatory and vasoconstrictive effects. When the phospholipid is a phosphatidylcholine, a lysophosphatidylcholine (LPC) is formed, which may interact with cells via G-protein-coupled receptor-mediated or direct membrane interactions, or may undergo further enzymatic modification to form other bioactive lipids.
spermatogenesis and inflammation in the testis (Ishikawa and Morris 2006; Ishikawa et al. 2005). In addition, PGE2 and PGF2α, produced by mature spermatozoa play a role in the acrosome reaction (Breitbart et al. 1995; Joyce et al. 1987). Products of the lipoxigenase pathway, such as leukotriene B4, are also produced by the testis, where they intervene in the regulation of Leydig cell steroidogenesis by LH (Papadopoulos et al. 1987; Sullivan and Cooke 1985), but other functions can be expected.

Direct evidence for the anticipated roles of prostanoids or lipoxygenase products in testicular inflammation and immunity is lacking. Initially, there was some evidence to suggest that PGE2 might be responsible for the immunosuppressive phenotype of resident testicular macrophages (Kern and Maddocks 1995; Kern et al. 1995). This now seems unlikely, because testicular macrophages produce little PGE2 under normal conditions, and blocking COX2 activity in vivo or in vitro does not affect the production of proinflammatory cytokines in the testis in response to LPS (Gerdprasert et al. 2002a; Winnall et al. 2007, 2008). In the rat, chronic inhibition of COX2 inhibited interstitial fluid formation in normal testes, but ameliorated the loss of fluid that usually occurs during LPS-induced inflammation (Winnall et al. 2008). The latter data would seem to indicate roles for products of COX2 in the control of vascular tone in the normal and inflamed testis and this deserves further exploration.

When phosphocholine (PC)-containing phospholipids are used as the substrate for PLA2 action, lyso phosphatidylcholines (LPCs) are also produced (Aoki et al. 2002; Snyder 1990). LPCs usually comprise a glycerophosphocholine backbone, a single long-chain fatty acid, and a free hydroxyl group (Khaselev and Murphy 2000). The platelet-activating factors (PAFs) are a subset of LPCs with an acetyl group in place of the hydroxyl group, which facilitates binding to the PAF receptor (Snyder 1990). The effective concentrations of these phospholipid-derived molecules in biological fluids are normally tightly controlled by binding to albumin and lipoproteins (Croset et al. 2000), since some LPCs, particularly those derived from the saturated C16 and C18 fatty acids, cause cell lysis at high concentrations (Weltzien 1979). At physiological concentrations, however, LPCs induce specific responses in various cell types, including most immune cell types. Nonacyetylated LPCs are chemotactic toward macrophages, T cells, and NK cells, and stimulate COX2 expression by macrophages, cytokine production by T cells, and the cytotoxic activity of NK cells (Légrádi et al. 2004; Radu et al. 2004; Whalen et al. 1999; Yang et al. 2005). In addition, LPCs induce apoptosis in T cells, indicating a role in immunosuppression as well (Kabarowski et al. 2002; Takeda et al. 1982). The details of the mechanisms involved are poorly understood. While there is evidence for involvement of specific G-protein-coupled receptors (Flemming et al. 2006; Lin and Ye 2003; Radu et al. 2004; Soga et al. 2005; Yang et al. 2005), LPCs may interact with G-proteins, membrane kinases, and ion channels directly, thereby bypassing the need for any specific receptor-ligand interactions.

The interstitial fluid of the testis has long been known to possess potent lymphocyte-inhibiting activity in vitro (Hedger et al. 1998a; Pöllänen et al. 1988). The molecules responsible for this activity have now been isolated and identified as the specific LPCs: 1-palmitoyl-sn-glycero-3-phosphocholine (16:0a-LPC), 1-oleoyl-sn-glycero-3-phosphocholine (18:1a-LPC), a 18:2a-LPC (putatively, 1-linoleoyl-sn-glycero-3-phosphocholine), and a 20:4a-LPC (putatively, 1-arachidonoyl-sn-glycero-3-phosphocholine) (Foulds et al. 2008). These molecules inhibit T cell proliferation in response to activation and induce apoptosis of these cells in a time- and dose-dependent manner at physiologically relevant concentrations. Although PAF activity has also been found in the testis (Muguruma et al. 1993), these acetylated LPCs did not appear to contribute significantly to the T cell inhibitory activity. The emergence of LPCs as regulators of critical immune events in the testis opens up new avenues of inquiry into the origins of autoimmune infertility and more general mechanisms of peripheral immunoregulation. These molecules may also have other roles in testis biology that demand consideration.

11.10.4.3.5 Cell surface regulatory molecules: Complement inhibitors and nonclassical MHC

Inhibition of complement activation is an important mechanism for reducing transplant rejection, particularly in the early phase. Membrane complement inhibitors, such as CD46, CD55, CD59, and the complement receptor-related protein (Crry), are expressed in several immune-privileged tissues and have been implicated in suppression of inflammation in the eye and placenta (Bora et al. 1993; Bulla et al. 2005). CD46 and CD55 appear to be confined to spermatids in the adult rat testis, Crry is expressed
on early spermatocytes and interstitial cells, but CD59 is widely expressed throughout the seminiferous epithelium and interstitium (Mizuno et al. 2006). A role for CD59 expression by the Sertoli cells in prolonging the survival of these cells in transplantation studies has been suggested (Lee et al. 2007). Moreover, complement regulators both in seminal plasma and on the surface of the spermatozoa (CD46, CD55, and CD59) may contribute to complement evasion by the spermatozoa in the female tract (Harris et al. 2006).

Classical MHC class I molecules (HLA-A, HLA-B, and HLA-C) are expressed on nearly all cell types, and facilitate antigen presentation by these cells and activation of cytotoxic T cells (Cresswell et al. 1999; Janeway 1992; Sant et al. 1999). By contrast, the non-classical MHC class Ib molecules (HLA-E, -F, and -G) are much less widely expressed, and are associated with suppression of the adaptive immune response. Membrane-bound and soluble variants of these MHC class Ib molecules have been implicated in the apoptosis of alloreactive cytotoxic T cells, inhibition of NK cell cytolytic activity, suppression of T cell proliferation, and deviation of type 1 to type 2 responses, most notably in pregnancy (Bainbridge et al. 2000; Fournel et al. 2000; Ishitani et al. 2006; Kapasi et al. 2000; Kanai et al. 2001; Riteau et al. 1999). HLA-G has been found in the rhesus monkey testis (Ryan et al. 2002; Slukvin et al. 1999), and HLA-E is expressed on germ cells in the human testis (Fiszer et al. 1997), indicating that these molecules may contribute to immune privilege in the testis.

### 11.10.4.4 Immune System–Testis Interactions in Normal Function and Pathology

From the evidence outlined in the previous sections, it should be evident that proinflammatory and immunoregulatory processes are involved in both normal testicular function and testicular pathophysiology. In the normal testis, roles in the maintenance of immune privilege, the control of Leydig cell development, and the fine regulation of spermatogenesis are indicated (Figure 4). Contributions to pathology include the development of autoimmunity within the

![Figure 4](image-url)
male reproductive tract, disruption of steroidogenesis and spermatogenesis during systemic and local inflammatory events, and the response to vascular disturbances.

11.10.4.4.1 Normal testicular function

Direct evidence that immune privilege is due to a specialized immune environment originally comes from studies involving the central nervous system. Injection of soluble antigen into the compartments of the eye or brain produces antigen-specific suppression of cell-mediated immunity, specifically type 1 reactions (Kaplan and Streilein 1978; Wenkel et al. 2000). This phenomenon is called acquired immune deviation (AID), and it is possible to elicit similar inhibition of antigen-specific immune responses by prior injection of antigens into the testis (Ditzian-Kadanoff 1999; Li et al. 1997; Veräjänkorva et al. 2002). The Sertoli cell and Leydig cell are almost certainly central to this process, although contributions from other testicular cells are to be expected. The mechanisms responsible presumably involve the recruitment and functional deviation of macrophages (and possibly dendritic cells) within the testis (Hedger 2002), the subsequent recruitment of immunoregulatory lymphocyte subsets (Tompkins et al. 1998), the production of immunoregulatory cytokines, such as TGFβ family members and IL10 (O’Bryan et al. 2005; Suarez-Pinzon et al. 2000), local high concentrations of androgenic steroids, prostanoids, and other bioactive lipids (Foulds et al. 2008; Winnall et al. 2007), complement inhibitors (Lee et al. 2007; Mizuno et al. 2006), and testicular expression of inhibitory MHC and costimulatory molecules (Dal Secco et al. 2008; Fiszer et al. 1997; Ryan et al. 2002).

Macrophages play an important role in Leydig cell development and function. There is a close temporal link between the maturation of the adult Leydig cell population and the increase in the number of testicular resident macrophages during puberty (Hardy et al. 1989; Raburn et al. 1993; Vergouwen et al. 1993). Reductions in intratesticular macrophages due to specific depletion methods or in the macrophage-deficient (op/op) mouse lead to disorders of Leydig cell development and mature function (Cohen et al. 1997; Gaytan et al. 1994a,b), and testicular macrophage-conditioned medium stimulates Leydig cell steroidogenesis under certain conditions (Nes et al. 2000; Yee and Hutson 1985). These supportive functions of the testicular macrophages may involve production of cytokines and other secreted mediators (Khan et al. 1992b; Warren et al. 1990), as well as the distinctive intercytoplasmic specializations that develop between the two cell types (Hutson 1992; Miller et al. 1983). It has also been shown that testicular macrophages support basal steroidogenesis in the Leydig cells by providing 25-hydroxycholesterol as a substrate for testosterone biosynthesis (Nes et al. 2000).

Endogenous regulation of inflammatory pathways within the seminiferous epithelium appears to be involved in the fine control of spermatogenesis. Studies in the rat and mouse indicate that nuclear localization (i.e., activation) of the key proinflammatory transcription factor NFκB, and expression of the inflammation-responsive genes, IL1α, TNFα, IL6, activin A, and iNOS, in the Sertoli cell and the germ cells describe cyclical patterns within the seminiferous epithelium that coincide with critical events in the cycle of the seminiferous epithelium (O’Bryan and Hedger 2008). Most notably, release of spermatozoa from the epithelium (spermatiation) in the rat testis is followed by an increase in the production of IL1α, IL6, and activin A by the Sertoli cells and of TNFα and iNOS by the spermatocytes (De et al. 1993; Hakovirta et al. 1995; Kaipia et al. 1992; Söder et al. 1991; Syed et al. 1993; O’Bryan et al. 2000a; Okuma et al. 2006). In the mouse, spermiation is accompanied by a period of elevated nuclear NFκB levels in spermatocytes (Delfino and Walker 1998). These responses coincide with a peak of DNA synthesis by preleptotene spermatocytes and type A spermatogonia prior to meiotic and mitotic division, and reorganization of Sertoli cell tight junctions to allow the meiotic cells to enter the adluminal compartment (O’Bryan and Hedger 2008). Given that IL1α, IL6, and activin A are regulators of spermatogonial proliferation and meiotic progression (Hakovirta et al. 1993a, 1995; Mather et al. 1990; Meehan et al. 2000; Meinhardt et al. 2000; Parvinen et al. 1991; Söder et al. 1991), while TNFα and iNOS/NO induce the disassembly of the intercellular tight junctions (Lee and Cheng 2003; Lee et al. 2005; Li et al. 2006a; Siu et al. 2003), this concurrence of events is unlikely to be a coincidence. It would appear that release of the spermatozoa and/or phagocytosis of the residual cytoplasm triggers elements of the inflammatory machinery within the seminiferous epithelium. A role for TLRs or other pattern-recognition receptors in this process may be postulated, and similar regulatory networks may also operate throughout the remainder of the cycle. Many questions regarding the details of this functional regulation await clarification, and it must be
noted that there is a curious lack of concordance between the content of NFκB in the Sertoli cell nucleus observed across the cycle in the mouse (Delfino and Walker 1998) and inflammatory cytokine production by these same cells in the rat (De et al. 1993; Hakovirta et al. 1995; Kaipia et al. 1992; O’Bryan et al. 2000a; Okuma et al. 2006; Söder et al. 1991; Syed et al. 1993). Regardless of the questions that remain, however, key elements of the inflammatory response appear to play essential roles in the physiological regulation of the seminiferous epithelium, which are entirely separate from their roles in pathology.

11.10.4.4.2 Testicular pathology
Although it is a common assumption that male reproductive failure caused by local or systemic illness, infection, and chronic inflammatory disease is due to the negative effects of raised body temperature on spermatogenesis, there is little clinical or experimental evidence to support this belief (O’Bryan et al. 2011; Syed et al. 2001; Starace et al. 2005). There is much more convincing evidence that the damage is due to activation of local inflammatory pathways and the activity of immune cells recruited during inflammatory events. As already outlined in the previous sections, inflammatory mediators such as IL1, ROS, TNFα, and NO, as well as glucocorticoids produced in response to inflammation, have mostly negative effects at all levels of the hypothalamic–pituitary–Leydig cell axis (Bambino and Hsueh 1981; Del Punta et al. 1996; Hales 1992; Hales and Payne 1989; Hardy et al. 2005; Li et al. 1995; Mauduit et al. 1991b, 1998; Monder et al. 1994; Sharma et al. 1998; Welch et al. 1993; Xiong and Hales 1993b, 1994, 1997). The subsequent reduction in androgen production may lead to reduced spermatogenic capacity, but may also exacerbate the effects of inflammation on spermatogenesis itself. Expression of TLRs on the Sertoli cells is obviously important in protective responses against pathogens, but also provides a mechanism whereby pathogens can directly inhibit spermatogenesis. Thus, TLR activation and the production of inflammatory mediators almost certainly alter Sertoli cell functions, and this will affect the ability of these cells to support and regulate spermatogenesis, as well as activate signaling pathways that promote germ cell apoptosis (Lysiak et al. 2005; Pentikäinen et al. 2002; Rasoulpour and Boelkelheide 2005; Starace et al. 2005).

Inflammatory events play a role in testicular damage due to vascular disturbance. Spermatogenesis is an energy intensive process, and the seminiferous epithelium is not vascularized. Consequently, unobstructed blood flow to the testis is essential and even minor anomalies in the testicular vasculature, such as varicocele, have cumulative negative effects on sperm production (Jarow 2001). The deleterious effects of cadmium on fertility through disruption of the testicular vasculature are well known (Schrag and Dixon 1981). In experimental animals, transient torsion of the spermatic cord that renders the testis ischemic followed by reperfusion causes an increase in TNFα and IL1β expression, activation of the stress-related Jnk/p38 MAP kinase signaling pathway, neutrophil recruitment and infiltration into the testis, increased oxidative stress, germ cell apoptosis, and significantly decreased serum testosterone levels (Lysiak 2004; Lysiak et al. 2001; Rodriguez et al. 2006). A specific role for testicular nitric oxide in mediating damage in both the testicular torsion and human varicocele models has been implicated (Moon et al. 2005; Santoro et al. 2001; Shiraishi and Naito 2007; Shiraishi et al. 2001; Türker Köksal et al. 2004).

Administration of high doses of human chorionic gonadotropin (hCG), a treatment used to correct delayed testicular descent in young boys, causes a hyperstimulation syndrome in rats comprising increased testicular blood flow and pressure, increased vascular permeability, and accumulation of interstitial fluid (Hjertkvist et al. 1993; Setchell and Sharpe 1981; van Vliet et al. 1988). These vascular changes are accompanied by accumulation of intravascular and interstitial neutrophils in the testis (Bergh et al. 1986; Widmark et al. 1987), and spermatogenic damage (Kerr and Sharpe 1989a,b). The responses to hCG can be eliminated by depletion of either the Leydig cells or the neutrophils (Setchell and Rommers 1985; Widmark et al. 1987), and IL1β is able to replicate most of the effects (Bergh and Söder 1990; Bergh et al. 1996), suggesting that the response involves an inflammatory event mediated via IL1β secreted by the Leydig cells. Thus, it appears that disruption of the seminiferous epithelium in both the ischemia/reperfusion model and hCG hyperstimulation models is directly linked to local recruitment of neutrophils. Exactly how these cells exert damage on the germ cells is not yet known, but increased oxidative stress is obviously involved. The responses are quite different from those observed in LPS-induced inflammation, which paradoxically causes a large reduction in interstitial fluid volume and an increase in mononuclear cells in the rat testis (O’Bryan et al. 2000b; Gerdprasert et al. 2002a,b), although the germ cell damage is similar in both models.
In summary, different testicular inflammation models have direct effects on spermatogenic development, in addition to causing damage to the steroiodogenic function of the Leydig cells. It should also be noted that, as in most other tissues, inflammation responses within the testis do not automatically lead to autoimmune complications, but damage to various testicular functions that may be less readily apparent may lead to longer-term consequences for reproductive health (Schuppe and Meinhardt 2005; Schuppe et al. 2008; Suominen and Soderstrom 1982).

11.10.4.5 The Epididymis, Vas Deferens, Accessory Glands, and Urogenital Tract

As already noted above, the immune environment of the remainder of the male tract is quite distinct from that of the testis, or even from that of other mucosal tissues. The mechanisms whereby sperm within the male reproductive tract are protected from the immune system remain largely unknown, but an intact tract is obviously important. In humans, vasectomy leads to sperm antibody formation in approximately 70% of cases, and sperm antibodies are also associated with obstructive azoospermia and congenital absence of the epididymis and/or vas (Alexander and Anderson 1979; de Kretser et al. 1998; Hellema et al. 1979; Patrizio et al. 1992). The incidence of antibody formation in humans appears to be directly related to the distance of the lesion from the testis, implying a limited role for elements of testicular immune privilege in preventing immune reactions. There is evidence from patients and animal models that vasectomy can have deleterious effects on the testis and epididymis as well, and that at least some of these effects may have an immunological basis (Airken et al. 1999; Bigazzi et al. 1976; Flickinger et al. 1990a; Raleigh et al. 2004). In certain strains of rats, mice, and rabbits, vasectomy rapidly leads to orchitis and sterility, so it is not clear why this degree of damage is so rare in humans. In reality, the immunology of the male reproductive tract has yet to be investigated in depth, but two areas of recent interest are worth highlighting: the TLRs and the defensins.

Given the exposure to the external environment, it is not surprising that the TLRs as well as many essential TLR-related genes, such as MyD88, are expressed throughout the male reproductive tract, including the epididymis, vas deferens, and accessory glands (Nishimura and Naito 2005; Palladino et al. 2007; Quintar et al. 2006; Rodrigues et al. 2008). Expression is found on both epithelial cells and leukocytes in these tissues. Moreover, weak expression of TLR11, originally localized to the urinary tract of experimental rodents, has been detected in the epididymis and vas deferens of the rat (Lauw et al. 2005; Palladino et al. 2007; Zhang et al. 2004). The importance of these molecules in acute and chronic inflammation, and the response to infection, in the male reproductive tract is an important area for future studies. Moreover, changes in susceptibility to inflammation through polymorphisms in the TLRs have been associated with differences in susceptibility to the onset of cancer in the prostate (Sun et al. 2005; Zheng et al. 2004).

Defensins are small (3–4 kDa) positively charged peptides that are able to disrupt bacteria, fungi, parasites, and some enveloped viruses by forming multimeric pores in the pathogen membrane (Selsted and Ouellette 2005). The β-defensins are produced by most mucosal epithelial tissues, including the testis and epididymis (Com et al. 2003), and their production is stimulated via TLR activation and cytokines. A number of epididymal-specific β-defensins have been identified in the mouse and the rat (Li et al. 2001; Jalkanen et al. 2005; Yamaguchi et al. 2002; Yenugu et al. 2004; Zhou et al. 2004), and a role for androgen in their regulation has been indicated (Jalkanen et al. 2005; Yenugu et al. 2004). Apart from their obvious role in protection against pathogens, defensins may have other functions in the reproductive tract. The novel β-defensin, Bin1b, which is exclusively produced and secreted by the rat caput epididymis, binds to sperm (Li et al. 2001; Zhou et al. 2004), and blocking Bin1b reduces sperm motility in vivo, suggesting a novel role for this molecule in sperm maturation (Zhou et al. 2004).

11.10.4.6 Sperm, Semen, Seminal Leukocytes, and Cytokines

In humans, semen is the most accessible window into the health of the male reproductive system, as it is the number and quality of sperm in the semen that provide the principal foci for male reproductive toxicity outcomes. However, semen is a complex secretion, comprising many components, including leukocytes, immunoglobulins, cytokines, prostaglandins, and various immunosuppressives. Changes in the number or activity of these immunological components of the semen are also potential indicators of toxic actions within the tract.
Elevated numbers of leukocytes in the semen are generally considered to be an indication of infection, but leukocytes are present even in the semen of men with normal fertility (Aitken and Baker 1995; Barratt et al. 1991). The origin of these cells is somewhat obscure. Evidence suggests that the epididymis or vas may be a major source (Anderson et al. 1991; Schwartz 1990), but the main leukocyte subset present in most semen samples are neutrophils, which are not a normal feature of the tissues of the genital tract (Aitken and Baker 1995; el-Demiry et al. 1986; Wolff and Anderson 1988). The impact of these cells on fertility is also poorly understood. Some studies have shown a relationship between leukocytospermia and impaired sperm function (Auroux 1984; Aziz et al. 2004; Berger et al. 2004; Maegawa et al. 1999; Rajasekaran et al. 1996). However, specific positive and negative associations with other forms of infertility have also been reported (Eggert-Kruse et al. 2001; Gruschwitz et al. 1996; Huleihel et al. 1999; Loras et al. 1999; Naz and Evans 1998).

Seminal plasma has profoundly immunosuppressive properties, as defined by the ability to inhibit various T cell and NK cell activities in vitro. This inhibitory activity has been attributed to a number of seminal factors, including prostasomes (Kelly et al. 1991), oxidized polyamines (Allen and Roberts 1986), prostaglandins of the E series (Skibinski et al. 1992), nonspecific lymphocyte-suppressing proteins (Maccioni et al. 2001; Veselský et al. 2002), and immunoregulatory cytokines (Anderson et al. 1998; Huleihel et al. 1999; Loras et al. 1999; Miller et al. 2002; Nocera and Chu 1993; Rajasekaran et al. 1996; Srivastava et al. 1996). The main cytokines with immunosuppressive activity in human seminal plasma are TGFβ1, TGFβ2, IL10, and activin A. Immunosuppressives are believed to play a role in preventing lymphocyte responses against sperm autoantigens in the male and female reproductive tracts (Robertson et al. 2003; Ochsenkuhn et al. 2006).

11.10.5 General and Toxicological Issues

In the current state of understanding of the interactions between the male reproductive tract and the immune system, many questions still remain. Nonetheless, it is clear that the mammalian testis in particular displays a special, if not unique, relationship with the immune system, involving distinctive cell–cell interactions and shared cytokines. Recently, microarray analysis of testicular gene expression signatures in human spermatogenic failure were found to correspond with gene sets usually associated with inflammation and autoimmune disease (Spiess et al. 2007). This relationship between reproduction and immunology impacts upon normal function, since perturbations in one system caused by toxicants will almost certainly impinge upon the other system. This extends into the characteristic responses of the testis.
to toxins and various pathologies (Figure 5). The complexity and potential consequences of these interactions may be appreciated by consideration of the following propositions:

1. **If leukocytes play a role in maintaining normal testicular function, then drugs and toxicants that reduce macrophage and lymphocyte function or numbers may also have an indirect effect on testicular function.** The effect of dichloromethylene diphosphonate on macrophages in the rat provides a good experimental example of this possibility, by demonstrating that the loss of functioning macrophages leads to a decline in androgen levels and retarded Leydig cell development (Bergh et al. 1993; Gaytan et al. 1994a). Inhibition of macrophage and lymphocyte functions or numbers may also compromise the immune-privileged status of the testis, potentially leading to testicular autoimmune disease. A related subset would involve chemicals that have direct action on both the immune system and testicular function, such as cyclosporin and cyclophosphamide (Cavallini et al. 1990; Meistrich 1984; Seethalakshmi et al. 1990; Wetzels 2004), which are commonly used to suppress graft rejection response and in the treatment of some autoimmune diseases.

2. **Drugs or toxicants that induce inflammation may interfere with normal testicular function.** The serious consequences for testicular function associated with immune activation and inflammation are illustrated by the experimental model of LPS administration, which leads to Leydig cell dysfunction and testicular failure (Christeef et al. 1987; Wallgren et al. 1993; O’Bryan et al. 2000b). Any drug or toxicant that induces macrophage activation (e.g., bacterial toxins, carbon monoxide, sulfur dioxide) has the potential to induce testicular production of inflammatory cytokines, such as IL1/β, TNFα, and IFNγ, as well as ROS, which may interfere with Leydig cell function and

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**Figure 5** Model of toxicant actions and potential immunological consequences in the testis. Toxicants may damage or alter the function of somatic cells (in particular the Sertoli and Leydig cells), immune cells, or germ cells, thereby inhibiting androgen production and spermatogenesis, leading to increased germ cell death. This damage may lead to an overt inflammatory event (orchitis) through activation of the proinflammatory activities of local macrophages, which may exacerbate or prolong the testicular dysfunction. These events may be followed by a full or partial recovery of normal function, but intratesticular immune cell numbers and activity may become permanently altered, with possible implications for future toxic or inflammatory episodes. In certain circumstances, which may depend upon the severity of the initial damage caused or the genetic background of the patient, the antigen-presenting activity of intratesticular dendritic cells or macrophages may also be activated. This may lead to recruitment and activation of testicular antigen-specific T cells, development of cytotoxic T cells (CTL), and B cell antibody (Ig) production. This subsequent autoimmunity may have a more permanent effect on testis or sperm function (epithelial destruction or sperm antibody formation).
spermatogenic development, or local immunoregulation. The danger hypothesis model of autoimmune disease may be particularly relevant in this instance (Matzinger 1994). At least one of the putative ‘danger’ molecules (i.e., TNFα) is endogenously produced in the testis, potentially presenting a continuous challenge to the testicular immune system.

3. Drugs or toxicants that induce autoimmune disease or allergy may also induce testicular autoimmune disease. There are many drugs that induce polysystemic autoimmune disease and this may also include reactions against the germ cells. An example here is polyarteritis nodosa, a necrotizing vasculitis of small arteries, which frequently involves the testis (Fauci et al. 1978). Sulfonamides, penicillin, phenytoin, arsenicals, thiouracil, iodides, and thiazides are frequently suspected as causes of this condition (Nusinow et al. 1985). Likewise, mutagens and other toxins that may alter T and B cell receptor repertoires to create self-reactive lymphocytes could also contribute to the onset of autoimmune disease in the male reproductive tract.

4. Drugs or toxicants that affect testicular cell development or viability may also induce immune responses. There are drugs or toxicants that affect Sertoli–Sertoli and Sertoli–germ cell junction stability, as well as agents that damage the Sertoli cell or germ cells directly, resulting in massive germ cell death, which may overwhelm the normal immunoregulatory capacity of the testis. Examples of this interaction are seen after testicular heating, serotonin administration, or treatment with ethane dimethane sulphonate (EDS) or other testicular toxicants, which are frequently followed by transient immune and/or inflammatory events (Creasy et al. 1983; Hedger et al. 1995; Padmanabhan and Singh 1981; Wang et al. 1994). Even if the epithelium recovers fully, there is the potential for sperm antibody development, leading to reduced fertility. Increased exposure to estrogenic compounds is associated with activation of immune cells in the testis, most notably the macrophages, providing evidence for a mechanism whereby environmental estrogens may affect male fertility indirectly via effects on local immunity (Li et al. 2006b).

5. Testicular toxicants that alter steroidogenic cell function will have a direct effect on the immune system. Toxicants that damage Leydig cells and reduce steroid production possibly could alter thymus function, leading to an enhanced susceptibility to autoimmune disease. Balanced against this is the likelihood that other androgen-deficiency symptoms are far more likely to be manifest and treated. All these interactions, however, illustrate to one degree or another the difficulty of separating direct effects of toxic agents on the immune cells and on the testis.

11.10.6 Conclusions

There is a dynamic relationship between the male reproductive tract and the immune system that should be considered when assessing the effects of various toxicants on male fertility and reproductive development. The effects of toxicants on male reproductive functions, most notably the production of androgenic steroids, will in turn impinge upon the functions of the immune system. Many mechanisms underlying these interactions have begun to emerge in recent years, but a complete picture remains hidden. Further studies will no doubt lead to important new concepts concerning this relationship and its implications for reproductive toxicology.

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