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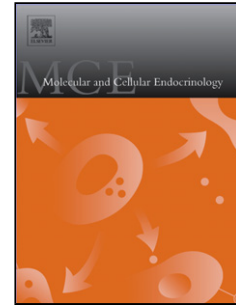
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Testicular innate immune defense against bacteria

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Abstract

Infection and inflammation are relevant entities of male factor infertility. Bacterial infections are mostly the consequence of an ascending infection of the genito-urinary tract which can ultimately lead to epididymo-orchitis. Bacterial toxins and the innate immune responses directed against them may have a significant impact on male reproductive function. Toll-like receptors (TLRs) constitute the major family of pattern recognition receptors that play a pivotal role in innate immunity. In the testis, TLRs are not only found in immune cells such as macrophages and dendritic cells, but also in testicular somatic cells and to a lesser extent in germ cells. In this review we describe relevant bacterial pathogens found in testicular and male reproductive tract infection, new data on the localisation and potential functions of TLRs, recognition and response to bacteria with a special emphasis on uropathogenic *E. coli*. Mechanisms by which uropathogenic *E. coli* subvert innate immune responses in the testis are discussed using information derived from animal model studies.

Relevant pathogens causing testicular dysfunction

Following some controversy in the past it is now accepted that bacterial and viral infections of the male reproductive tract contribute significantly to impaired fertility. Bacterial infection of the male genital tract often results from ascending canalicular infections of the male excurrent ducts and can manifest itself as urethritis, prostatitis/vesiculitis, epididymitis or epididymo-orchitis. Bacteria eliciting epididymo-orchitis are either sexually transmitted or originate from urinary tract infections (Purvis & Christiansen, 1993; Weidner et al., 1999; Anders & Patole, 2005; Ludwig, 2008). Among sexually active men younger than 35 years with a medical history of urethral discharge, the most prevalent pathogens related to epididymo-orchitis are *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (Weidner et al., 1999; Ludwig, 2008; Ochsendorf, 2008). Apart from sexually transmitted infections, the most common cause for epididymo-orchitis is genito-urinary tract infection with *E. coli* and other *Enterobacteriaceae* especially in older men. Uropathogenic *Escherichia coli* (UPEC) are among the most frequently isolated microbial agents in urinary tract infections, whereas in semen the subtype of *E. coli* is rarely specified (Wiles et al., 2008).

Bacterial epididymo-orchitis represents a relevant clinical entity in andrology and urology. A prevalence of 600,000 cases of acute epididymitis per year has been reported in the USA (Krieger, 1984). According to Collins et al. (Collins et al., 1998) epididymitis/orchitis accounted for 0.30% of all consultations to office-based physicians of all specialties in the USA, and was ranked fifth among genitourinary diagnoses in those aged 18-50 years. In a recent urological outpatient study, epididymitis accounted for approximately 1% of visits, with 80% of these patients having symptoms longer than 3 months (Nickel et al., 2002). Inflammation of the testis as a complication of acute epididymitis due to ascending, canalicular bacterial infections is common and may occur in up to 60% of affected patients (Ludwig et al., 2008; Schuppe et al., 2008). Of note, whereas acute testicular inflammation is symptomatic, most patients with subacute or chronic impairment are asymptomatic (Schuppe et al., 2008). Treatment of the acute phase of disease, i.e. bacterial epididymo-orchitis, is based on antibiotic and antiphlogistic pharmacotherapy (Weidner et al., 2002). Even after successful intervention, however, the inflammatory reaction can chronify silently in both testes with major

functional and structural damage of the seminiferous tubules and subsequent infertility (Weidner et al., 1990; Osegbe, 1991; Fijak & Meinhardt, 2006; Schuppe et al., 2008). Hence, it is almost certain that genital tract inflammation/infection including the chronic form contributes to a larger extent to the cohort of patients with “idiopathic” infertility (Schuppe & Meinhardt, 2005; Haidl et al., 2008). Until now, however, the clinical identification of chronic genital tract inflammation/infection has been hampered by the lack of symptoms and non-invasive diagnostic tools.

Uropathogenic *E. coli*

E. coli, the most common facultative gram negative bacteria in the human fecal flora, usually resides in the colon as a harmless commensal. From a clinical point of view, *E. coli* of biological importance to humans can be broadly classified as (1) commensal *E. coli* (i.e. intestinal colonizers), (2) intestinal pathogenic *E. coli* (i.e. enteric or diarrheagenic strains), and (3) extraintestinal pathogenic *E. coli* (ExPEC) (Russo & Johnson, 2000). *E. coli* is a highly clonal species represented by 4 major phylogenetic groups comprising A, B1, B2 and D (Johnson & Russo, 2005). Diarrheagenic *E. coli* occur almost exclusively in A, B1 and D as well as other ungrouped phylogenetic groups, while uropathogenic and other extraintestinal *E. coli* are present within the B2 group. Uropathogenic *Escherichia coli* (UPEC) are the most frequent cause of urinary tract infection and are responsible for 70–90% of the anticipated 150 million urinary tract infections diagnosed annually (Stamm & Norrby, 2001). UPEC have acquired specific virulence attributes such as adhesins, α -haemolysin, siderophores and polysaccharide coatings (Eisenstein & Jones, 1988; Johnson, 1991; Johnson & Kuskowski, 2000; Johnson & Stell, 2000; Russo & Johnson, 2000). These factors confer upon UPEC the ability to avoid or subvert host defenses, colonize and invade host tissue, perturb host physiology, invade host tissues, and/or aggravate a deleterious host inflammatory response, thereby causing disease (Russo & Johnson, 2000). These UPEC virulence factors are usually encoded on the chromosome of UPEC and are often part of large, unstable chromosomal regions, known as pathogenic islands (PAIs) (Blum et al., 1994; Swenson et al., 1996; Hacker et al., 2003; Dobrindt et al., 2004). Currently nine PAIs have been described, but the majority of virulence determinants are located on only five

PAIs. Indeed, all virulent factors such as toxins, adhesins, an iron siderophore system, proteases, capsule and LPS are expressed on five PAIs present in UPEC CFT073 strain. The role in pathogenicity of these PAIs have been tested in a murine model. Mutational analyses of these PAIs have shown that deletion of a single PAI did not significantly affect the survival of infected mice. Deletions comprising two or three PAIs, however, significantly reduced the virulence potential of these strains (Brzuszkiewicz et al., 2006).

The two UPEC strains 536 and CFT073 have been completely sequenced and several of their PAIs have been studied in detail (Hacker et al., 1983; Mobley et al., 1990; Kao et al., 1997; Guyer et al., 1998; Rasko et al., 2001; Dobrindt et al., 2002; Schneider et al., 2004). The complete genome sequence analysis of UPEC strain CFT073 has revealed extensive mosaic structure in terms of the distribution of backbone gene conserved in *E. coli*, and “foreign” genes, which probably have been acquired horizontally (Welch et al., 2002). The genome *E. coli* strain CFT073 consists of a single circular chromosome of 5,231,428 bp and is 292 bp larger than that of strain 536.

Testicular innate immunity and defense mechanisms to bacterial infection

The excurrent ducts can provide a gateway for ascending microbial infections, which can manifest itself as urethritis, prostatitis, epididymitis or epididymo-orchitis. It is therefore not surprising that in 13-15% of all cases of male factor infertility infection and inflammation of the male genital tract are considered as primary cause or co-factor of fertility disturbances (WHO, 1987; Nieschlag & Behre, 2000; Dohle et al., 2005). On the other hand, the testis is an immunologically privileged organ protecting the auto-antigens of the meiotic and haploid germ cells, which first appear after the establishment of self-tolerance at the time of puberty (Head et al., 1983; Filippini et al., 2001; Hedger & Meinhardt, 2003; Fijak & Meinhardt, 2006). Immune privilege is attributed to tissues such as the eye, brain and fetal-maternal interface which prevent the spread of inflammation, because even minor incidences can threaten organ integrity and function (Girling & Hedger, 2007). It is therefore somewhat paradoxical that microorganisms can disrupt immune privilege causing impairment of spermatogenesis. At the cellular level testicular macrophages in the interstitial space, Sertoli cells and to a lesser extent peritubular cells have been implicated in both the maintenance of immune privilege and a

primary role in local inflammatory responses (Figure 1) (Fijak & Meinhardt, 2006). The direct pathologic effects resulting from the bacterial invasion are often aggravated by the inflammatory process (Schuppe & Meinhardt, 2005; Girling & Hedger, 2007). Consequently it is important to understand the mechanisms by which immunocompetent cells in the testis recognize infectious agents and how bacterial pathogens try to diminish an inflammatory response by interfering with testicular defence pathways (Figure 1 and 2).

Toll like receptors and innate immunity

All living beings are exposed constantly to microbial agents that are present in nature and need to deal with the invasion of these organisms into the body. The reliable detection of microorganisms is a complex and daunting task because of their molecular heterogeneity and rapid evolution. All vertebrates have developed immune responses to protect against potential microbial invasion. The vertebrate immune response can be functionally divided into innate and adaptive immunity, with the innate immunity being the first line of defense against microorganisms. Adaptive immune responses are on the contrary slower responses mediated by T and B cells which express highly diverse antigen receptors. Innate immunity had been considered as a nonspecific system, and until recently less attention has been paid towards the abilities of this system to contain insults. However, the discovery of pattern recognition receptors, which detect pathogens through evolutionary conserved pathogen-associated-molecular-patterns has greatly advanced the concept of innate immunity. Toll-like receptors (TLRs) and intracellular nucleotide-binding oligomerization domain proteins are two classes of pathogen recognizing receptors involved in innate immune detection.

The discovery of the TLRs was initiated by the finding of Toll, a gene product essential for the development of embryonic dorsoventral polarity in drosophila (Hashimoto et al., 1988). Subsequent studies have revealed that Toll plays a critical role in the antifungal responses of flies (Lemaitre et al., 1996). So far, 13 different members of the TLR family have been identified through data base search in mammals (10 in humans and 12 in mice) (Akira & Takeda, 2004; Beutler, 2004). TLR expression was found in various immune cells such as macrophages, dendritic cells, B cells, T cells, mast

cells, monocytes, neutrophils, eosinophil and even on non immune cells such as fibroblast and epithelial cells (Appelquist et al., 2002). Expression pattern of TLRs is not constant, but varies relatively quickly in response to the presence of their respective ligands. Among the characterized TLRs, TLR1, 2, 4, 5 and 6 are expressed on the cell surface and appear to exclusively identify bacterial and fungal components that are not made by the host, whereas TLR3, 7, 8 and 9, located in intracellular endosomes, specifically detect nucleic acids of pathogens (Akira et al., 2006).

The TLRs are type 1 integral membrane glycoproteins classified on the basis of considerable homology in the cytoplasmic domain to that of the interleukin type 1 receptor (IL-1R), termed the Toll/IL-1R homology domain (Bowie & O'Neill, 2000). The cytoplasmic domain of TLRs and IL-1Rs have a conserved region of approximately 200 amino acids, which is known as the Toll/IL-1R (TIR) domain (Slack et al., 2000).

Toll like receptor expression in the testis

The innate defence of the testis like that of other organs hinges on recognition of bacterial products by members of the Toll-like receptor (TLR) family. In view of the paradox immune status of the testis with its obvious susceptibility for infection and inflammatory responses in an immune privileged environment (Fijak & Meinhardt, 2006), it is surprising that only few studies have examined the role of TLRs in this organ thus far (Girling & Hedger, 2007). It is only recently that expression of TLR 1-11 was shown in total rat testis, epididymis and vas deferens (Palladino et al., 2007) and of TLR 1-9 in human testis samples (Nishimura & Naito, 2005). Riccioli et al. (Riccioli et al., 2006) reported expression of TLR 2-6 mRNA in mouse Sertoli cells. Stimulation of TLR2, 3 and TLR5 by agonists resulted in increased ICAM-1 and MCP-1 expression as well as the activation of the NF κ B pathway adding further credential to a central role of the Sertoli cells in testicular immunology (Riccioli et al., 2006; Starace et al., 2008).

In our own studies, a comprehensive expression analysis of TLRs and the adaptor molecule MyD88 in rat testicular cells by RT-PCR showed that testicular macrophages and dendritic cells express the mRNAs of all TLRs, while germ cells and somatic cells were usually positive for varying sets of two to three TLRs. Notably, TLR3 expression was most prominent in all testicular cells, except Leydig cells and spermatids, where only

weak expression was evident (Bhushan et al., 2008). This indicates a strong capacity of recognizing double stranded viral RNA in the testis. These results are largely in agreement with TLR expression in total testis of other species as well as in rat (Nishimura & Naito, 2005; Rodriguez-Martinez et al., 2005; Palladino et al., 2007), even though we could barely detect TLR4 and TLR6 synthesis in rat Sertoli cell as previously reported for the mouse by Riccioli et al. (Riccioli et al., 2006). The broad distribution of TLRs in the testicular cells indicates that they provide wide range detection of bacteria and viruses and play an important role in the innate immune system of the testis (Figure 1) (Girling & Hedger, 2007).

Toll like receptor signaling pathways

The engagement of TLRs by microbial agents activate a common signaling pathway that culminates in the activation of nuclear factor- κ B (NF κ B) transcription factors, as well as the mitogen-activated protein kinases (MAPKs) extracellular signal regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK). TLR ligand binding induces receptor dimerization and conformational changes which are required for the recruitment of the adaptor molecule to the TIR domain. Four TIR domain containing adaptor molecule have been identified to date; i.e. myeloid differentiation primary-response protein 88 (MyD88), MyD88 adaptor like protein (MAL), TIR-domain containing adaptor protein inducing IFN β (TRIF) and TRIF related adaptor molecule (TRAM) (Oshiumi et al., 2003; Yamamoto et al., 2003). Recently, a fifth adaptor molecule, sterile α and armadillo motif containing protein (SARM) has been identified, which negatively regulates TLR signal transduction (Carty et al., 2006). MyD88 is the key signaling adaptor molecule for all Toll like receptors with the exception of TLR3 and certain TLR4 ligands, which activates signaling through TRIF adaptor molecule. The respective TLRs signaling pathway involved is broadly classified into MyD88 dependent signaling pathway or MyD88 independent signaling pathway (Figure 2).

The MyD88-dependent pathway is similar to IL-1 receptors signaling pathways and the cytoplasmic domain of TLRs and IL-1 receptors share a common region which is

therefore termed Toll/IL-1 receptor (TIR) domain. The TIR signaling domain is instrumental in TLR signaling as upon binding to the ligand, MyD88 is recruited to the TIR domain of TLRs. MyD88 binding results in a signaling cascade which finally mediates ubiquitination and degradation of I κ B α via activation of various kinases and other intermediates. Subsequently, NF κ B translocates to the nucleus and activates expression of multiple pro-inflammatory cytokines such as archetypical IL-1, IL-6 and TNF α (Figure 2).

To activate the MyD88-independent signaling pathway, the N-terminal region of the TRIF adaptor molecule interacts with the TRAF family-member-associated NF- κ B activator (TANK) binding kinase 1 (TBK1) via TRAF3. Activated TBK1 in concert with inducible I κ B kinase (IKK α also called IKK ϵ) and two noncanonical IKKs phosphorylate IRF3 (Fitzgerald et al., 2003; Sharma et al., 2003). Phosphorylated IRF3 form homodimers which translocate into the nucleus, resulting in the expression of a set of IFN-inducible genes such as IFN α/β , IP10 and RANTES (Figure 2).

Impairment of testicular and sperm function by *E. coli*

The effects of *E. coli* on the male genital tract have been investigated to some extent in experimental and clinical studies. There are several *in vivo* as well as *in vitro* studies showing a negative effect of *E. coli* on human sperm function such as motility and acrosome reaction (Auroux et al., 1991; Merino et al., 1995; el-Mulla et al., 1996; Huwe et al., 1998; Kohn et al., 1998; Diemer et al., 2003). A direct inhibitory effect of *E. coli* on progressive motility was found to depend on bacterial concentration, and analysis by electron microscopy revealed multiple adhesions of *E. coli* to spermatozoa with a variable degree of ultrastructural damage present (Diemer et al., 1996). It has been shown that *E. coli* attaches to the superficial structures of human spermatozoa via type 1 adhesion molecules present on both bacterial pili and spermatozoa, respectively, that can be inactivated by preincubation with mannose (Wolff et al., 1993; Diemer et al., 1996; Diemer et al., 2000). Bacterial adhesion resulted in ultrastructural alterations and damage of the plasma membrane including the sperm head and acrosome. Defects in the acrosome, the mid piece, and the sperm tail apparently result in diminished motility and impaired fertilizing capacity of the affected gametes (Diemer et al., 2003). Oxidative

stress and apoptosis of spermatozoa was also observed following incubation with *E. coli* (Villegas et al., 2005; Fraczek et al., 2007). In rat *in vivo* models the retrograde inoculation of *E. coli* into the vas deferens promoted clinically recognizable acute orchitis characterized by severe degeneration of germinal epithelial cells, tubular atrophy, moderate inflammation and mild interstitial fibrosis with subsequent testicular damage (Demir et al., 2007). These findings are associated with a decrease in testicular volume and sperm concentration in comparison to control testes (Ludwig et al., 2002; Demir et al., 2007).

Uropathogenic *E. coli* (UPEC) subvert testicular immune response

Uropathogenic *Escherichia coli* (UPEC) have been shown to evade host innate immune response by inhibiting NF- κ B activation in testicular cells and urothelial cells ultimately leading to suppression of cytokine secretion (Bhushan et al., 2008; Billips et al., 2008). In clinical UPEC isolates 15 out of 17 strains revealed this ability (Billips et al., 2007). Many studies have shown that virulence factors of pathogens induces NF κ B suppression in host cells by targeting multiple signaling pathways and limit the host cell inflammatory responses (Ruckdeschel et al., 2001; Collier-Hyams et al., 2002; Yoshimura et al., 2002). *Yersinia pestis* injects a virulence factor (YopJ) in the host cell and blocks NF κ B activation and MAP kinase signaling by binding and preventing the activation of the upstream MAP kinases (MKKs) and IKK β kinases (Orth et al., 1999; Orth et al., 2000). Simultaneously, YopJ can suppress the expression of cytokines while inducing apoptosis during infection. Our work and that of others suggest that UPEC may also produce a virulence factor which is similar to YopJ in its ability to suppress the NF κ B signaling pathway. This hypothesis is supported by the fact that the suppressive capability required UPEC to be viable, as heat-killed bacteria failed to suppress epithelial responses (Hunstad et al., 2005).

By using *in silico* bioinformatics a hitherto uncharacterized open reading frame harbouring a Toll IL1R (TIR) domain has been documented in the UPEC CFT073 genome (Cirl et al., 2008). Further analysis revealed that the TcpC protein encoded in the SerU island of the UPEC strain CFT073 contains a TIR domain in the C-terminal region.

The TIR domain is universal to TLRs and required for docking of adaptor proteins MyD88, Mal and TRIF to trigger signal transduction such as MAPK activation. Interestingly, during the infection of kidney cells TcpC impaired this TLR signalling pathways by direct binding to MyD88 resulting in suppressed proinflammatory cytokine secretion (Cirl et al., 2008).

Sertoli cells, peritubular cells and testicular macrophages, all centrally implicated in the testicular defense system were found to express TLR4 (Bhushan et al., 2008) (Figure 1). TLR4 is the sensor of LPS, a major component of gram-negative bacteria cell wall. It is therefore surprising that incubation with UPEC or commensal non-pathogenic *E. coli* (NPEC) did not result in the release of the proinflammatory cytokines IL-1 α , IL-6 and TNF α despite of the partial activation of the MyD88-dependent TLR4 signaling pathway which is known to lead to their synthesis. Our data suggest that the lack of proinflammatory cytokine production is likely due to active suppression of the MyD88-dependent NF κ B pathway by UPEC at various levels of the signaling cascade depending on the cell type infected (Bhushan et al., 2008). All four examined cell types (Sertoli cells, peritubular cells and testicular and peritoneal macrophages, Figure 1) respond to incubation with UPEC, but not with NPEC, with elevated TLR4 protein levels. In peritubular cells and peritoneal macrophages as control downstream signaling after UPEC exposure is initiated as MAP kinases p38 and/or JNK are activated. These two kinases normally trigger transcription of proinflammatory genes including IL-1, IL-6 and TNF α by activation of nuclear transcription factors AP1 and NFAT. Stimulation of MyD88 pathway in peritubular cells and peritoneal macrophages culminates in degradation of I κ B α with subsequent transfer of p65 unit of NF κ B to the nucleus (Bhushan et al., 2008). In spite of activation of both MAP kinases and NF κ B the transcription of proinflammatory genes is blocked. In Sertoli cells and testicular macrophages the MAP kinases are activated, but not the NF κ B pathway, thus supporting the observation that lack of proinflammatory cytokine secretion is not related to lack of pathogen recognition, but more likely to virulence factors that block downstream transmission of proinflammatory signals. It seems that in testicular macrophages and Sertoli cells, NF κ B activation is already blocked before or at the point of activation of I κ B kinases. In PM and PTC however the MyD88 – NF κ B dependent cytokine

production must be abrogated at the start of gene transcription or at the posttranscriptional level (Bhushan et al., 2008).

Further support for an UPEC induced cytokine suppressing mechanism in the testis is derived indirectly from our observation that co-incubation of peritoneal macrophages with UPEC fully inhibited LPS or NPEC induced production of IL-1 α , IL-6 and TNF α (Figure 2). Similarly, IL-6 synthesis was found abrogated by UPEC strain UTI89 in bladder epithelial cells (Hunstad et al., 2005) with inhibition of the classical NF κ B pathway, albeit without evidence at which point this may occur (Klumpp et al., 2001). Unlike PM, all testicular cells were unresponsive to both LPS and NPEC challenge in terms of IL-1, IL-6 and TNF α secretion, however, we could detect upregulation of MCP-1, TGF β and COX-2 at least in TM.

Recent analysis of TLR4 signaling has revealed that LPS can induce a similar response as seen by stimulating TLR3, which senses double stranded viral RNA, by producing type I IFN such as IFN- α , IFN- β and IFN-response genes like MCP-1, IFN- γ -inducible protein 10 (IP10) and RANTES (Kawai et al., 2001). This so-called MyD88-independent pathway involves another adapter molecule TRIF which activates the TRIF-related adapter molecule (TRAM), causing phosphorylation of interferon regulated factor (IRF)-3 in infected cells. After transfer to the nucleus phosphorylated IRF-3 induces an antiviral and apoptotic response in various cells that is characterized by the production of endogenous type I IFNs and chemokines (Han et al., 2004; Ruckdeschel et al., 2004; De Trez et al., 2005; Kaiser & Offermann, 2005; Rasschaert et al., 2005; O'Neill & Bowie, 2007). This demonstrates that upon initial triggering by a pathogen, anti-viral and anti-bacterial pathways can merge by using common adapter molecules. In fact, infection of rat testicular somatic cells with Sendai virus, closely related to the mumps virus causing orchitis in humans, resulted in elevated levels of MCP-1, IP10 and IFN α mRNA (Dejucq et al., 1995; Le Goffic et al., 2002), a response very similar to that after UPEC challenge in this study. In the process of investigating a potential activation of the TLR4-TRIF/IRF3 pathway we found MyD88 independent nuclear translocation of IRF3 in all testicular cell types after treatment with UPEC, but not with NPEC (Bhushan et al., 2008). Furthermore, upregulation of IP10, RANTES and MCP-1 in peritubular cells as well as of IFN α/β and IP10 in testicular macrophages and Sertoli cells clearly indicated that

UPEC infection fully activated the TRAM-TRIF-IRF-3 alternative pathway resulting in transcription of these genes. This upregulated synthesis of chemokines may provide an explanation for the observed influx of leukocytes in bacterial orchitis (Mukasa et al., 1995).

In summary, UPEC-dependent inhibition of the cellular NF κ B pathway suppresses production of proinflammatory cytokines in the testis. However, subsequent inflammation is subverted by effective activation of the MyD88-independent pathway resembling an anti-viral immune response, thus promoting bacterial survival and virulence. In view of the immune privileged status of the testis, this analysis provides mechanistic insight into the process by which cytokine secretion is abrogated at different stages in the signalling pathways in different cell populations of the testis.

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Legend Figure 1

Simplified schematic representation of the testicular immune environment. A cross-section of the seminiferous epithelium consisting of Sertoli cells (SC) and germ cells (GC) with surrounding peritubular cells (PTC) is visible. The adjacent interstitial tissue contains the testicular leukocytes, amongst which monocytes/macrophages ($M\Phi$) are the most abundant immune cell type followed by T cells and NK cells. Mast cells (MC) are found in rodents only in small numbers under the capsule, but are more frequent in human where they are interspersed throughout the interstitial space. Dendritic cells (DC) together with monocytes/macrophages serve as 'professional' antigen-presenting cells which can incorporate and process antigen for presentation to CD4+ regulatory or 'helper' T cells. Bacterial infections are usually ascending canalicular reproductive tract infections. Within the lumen of the seminiferous epithelium bacterial pathogens are in direct contact to haploid germ cells and Sertoli cells. During infection bacteria can pass through the blood-testis barrier (BTB) or intracellularly and reach the PTC and interstitial cells. Binding of conserved microbial molecular patterns such as lipopolysaccharide to Toll like receptors triggers the innate immune response.

Legend Figure 2

Simplified model of the Toll-like receptor 4 (TLR4) signalling pathway. Bacterial lipopolysaccharide (LPS) triggers common TLR signaling through an adaptor protein, myeloid differentiation primary response gene 88 (MyD88), that recruits other intermediate molecules, which in turn activate mitogen activated protein (MAP) kinases such as p38, JNK and ERK1/2. Furthermore, NF κ B is translocated to the nucleus, where it directs transcription of the TLR-response cytokines IL-1, IL-6, TNF α . In addition to the common MyD88-dependent pathway, TLR 3 and TLR4 can utilize an alternative MyD88-independent signaling pathway where nuclear translocation of the transcription factor IFN-regulated factor (IRF-3) activates the expression of genes typical for anti-viral responses such as interferon (IFN) α/β and interferon γ induced protein (IP)-10. UPEC=uropathogenic E. coli.

