INVITED REVIEW

Sperm Biology

Epididymitis: revelations at the convergence of clinical and basic sciences

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Acute epididymitis represents a common medical condition in the urological outpatient clinic. Mostly, epididymitis is caused by bacterial ascent through the urogenital tract, with pathogens originating either from sexually transmitted diseases or urinary tract infections. Although conservative antimicrobial therapy is possible in the majority of patients and is usually sufficient to eradicate the pathogen, studies have shown persistent oligozoospermia and azoospermia in up to 40% of these patients. Animal models of epididymitis are created to delineate the underlying reasons for this observation and the additional impairment of sperm function that is often associated with the disease. Accumulated data provide evidence of a differential expression of immune cells, immunoregulatory genes and pathogen-sensing molecules along the length of the epididymal duct. The evidence suggests that a tolerogenic environment exists in the caput epididymidis, but that inflammatory responses are most intense toward the cauda epididymidis. This is consistent with the need to provide protection for the neo-antigens of spermatozoa emerging from the testis, without compromising the ability to respond to ascending infections. However, severe inflammatory responses, particularly in the cauda, may lead to collateral damage to the structure and function of the epididymis. Convergence of the clinical observations with appropriate animal studies should lead to better understanding of the immunological environment throughout the epididymis, the parameters underlying susceptibility to epididymitis, and to therapeutic approaches that can mitigate epididymal damage and subsequent fertility problems.

STRUCTURAL ORGANIZATION AND IMMUNE CELL DISTRIBUTION IN THE EPIDIDYMIS

The epididymal duct is lined by a pseudo-stratified epithelium, surrounded by a peritubular layer of smooth muscle cells, and an interstitial tissue stroma containing the vasculature and lymphatics. Principal cells comprise the main epithelial cell type and maintain the blood-epididymis barrier through apical intercellular tight junctions.[1] In many species, the epididymis is divided by connective tissue septa into morphologically and functionally distinct segments.[2] Functional segmentation also exists in the human epididymis, although physical segmentation is less evident.[3] Immune cells are found in all regions of the epididymis, and their distribution is highly organized. Macrophages and lymphocytes are frequently observed within the epididymal epithelium, where the latter are commonly called halo cells.[4] Most notably, the peritubular zone and epithelium of the caput epididymidis contain the greatest number of all immune cell types, which progressively decrease in number and apparent activity towards the cauda and vas deferens.[4–6] Conversely, the distribution of lymphatics in the epididymis appears to favor the caudal regions.[7]

Studies on immunity in the epididymis have been largely performed in rats and mice but are supported by available human data. Macrophages are the major epididymal immune cell, located chiefly in the interstitial and peritubular regions.[4,5] Macrophages in the epididymal stroma express major histocompatibility complex (MHC) class II antigens, required for antigen presentation to T cells, and MHC class II-restricted CD4^{+} T cells (helper and regulatory T cells) predominate over the CD8^{+} (cytotoxic) T cell subset, typical of the situation in other tissues.[5,8] Within the epididymal epithelium, by contrast, most macrophages lack MHC class II expression, and the majority of lymphocytes are CD8^{+} T cells, which is a common feature of mucosal epithelia.[4,5,8,9] Moreover, antigen-presenting dendritic cells form a dense network in the basal region of the epithelium and extend their processes toward the apical tight junctions between epithelial cells.[6] These dendritic cells appear to be particularly active within the proximal caput, where their processes may extend all the way through the epithelium.[6] They presumably sample antigens within the epididymal lumen and present them to the CD4^{+} T cells within the stroma and local lymph nodes, and could regulate antigen-specific immune responses to spermatozoa and pathogens. Given their known functions as regulators of immunity in other tissues, the intra-epithelial dendritic cells and macrophages can be expected to play a complex dual role in the epididymis, suppressing responses to sperm antigens under normal conditions, but activating responses to pathogens during infection.

Although basal cells, located adjacent to the basal lamina of the epididymis, exhibit some features typical of macrophages and can extend cytoplasmic processes towards the epididymal lumen, it appears

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that these cells are not macrophages or dendritic cells.[10] Their role in the epididymis remains unclear, but they may be involved in maintaining the blood-epithelium barrier, or function as luminal sensors to regulate the activity of other epithelial cells.

IMMUNOREGULATION IN THE EPIDIDYMIS

In contrast to the immunologically privileged testis, the epididymis does not appear to be able to support extended graft survival.[11] Furthermore, the epithelial tight junctions of the epididymis may not be as effective as those of the blood-testis barrier[1,12] and direct interactions between intra-epithelial immune cells and either sperm antigens, or ascending pathogens is possible. The epididymis certainly appears to be more susceptible to inflammation and autoimmunity than the testis, although proximity to the testis also appears to reduce the severity of these responses within the epididymis.[13–15] This indicates that the changing distribution of immune cells within the epididymis is reflective of a dynamically changing immunoregulatory environment.

The immunoregulatory enzyme, indoleamine 2,3-dioxygenase (IDO), is highly expressed in the caput epididymidis, and pro-inflammatory cytokine expression is increased in the caput epididymidis of IDO-deficient mice.[16,17] Levels of the immunoregulatory transforming growth factor-β (TGFβ) family cytokine, activin A, are also highest in this region.[18] Significantly, IDO is regulated through the SMAD2/3/4 signaling pathway that is activated by activin A,[19] suggesting that activin A may drive IDO expression in the proximal epididymis, and together these immunoregulatory proteins may induce a tolerogenic program in the intra-epithelial dendritic cell and T cell population. This would provide an effective mechanism for promoting tolerance to sperm antigens as they emerge from the immune-privileged environment of the testis. Stimulation of activin A and IDO in this region could involve androgens and other so-called "lumicrine" secretions from the testis, such as neurotropins, fibroblast growth factors, and the spermatozoa themselves, which are able to regulate the activity of cells in the proximal caput.[20,21]

The pathogen-sensing Toll-like receptors (TLRs) are expressed by the epithelial cells and in immune cells throughout the epididymis.[22] Levels of TLR1-6 in the caput epididymidis are similar to those found in the testis, with expression progressively declining towards the cauda and vas deferens. On the other hand, expression of TLR7, 9 and 11, as well as the TLR4 co-receptor CD14, tends to be higher in the epididymis than in the testis.[23,24] Changes in the distribution of these key regulators of innate immunity may have an influence on bacterial and viral pathogenicity in the different regions of the tract. Other innate immune system mechanisms are also regionally distributed along the length of the epididymis, such as the defensins, which are short peptides with potent antimicrobial activity.[25]

In summary, there is evidence that the differential expression of immunoregulatory genes, including IDO and activin A, and pathogen detection mechanisms, such as the TLRs, along the length of the epididymal duct leads to an environment where tolerogenic responses are more favored in the regions proximal to the testis, while antigen-specific immunity and inflammation responses are most vigorous in the cauda. This would be consistent with the need to protect sperm emerging from the testis, without compromising the ability to respond to ascending infections.

EPIDIDYMITIS IN MEN

Epidemiology

Acute epididymitis is a common condition with recent epidemiological data from the UK reporting incidence rates of about 25/10 000 person-years.[26] However, studies on the prevalence of epididymitis are scarce and subjected to specific population. In this context, epididymitis seems to be more common among military populations and individuals who have high-risk sexual behavior.[27] Nevertheless, acute epididymitis may affect patients at any age.[28–33]

Etiology

Acute epididymitis can be related to various etiologies (**Table 1**). Of these, pathogenic bacterial ascent through the urogenital tract is the most important cause. In 1927, by using an epidemiological approach, Campbell concluded that gonococcal epididymitis arose as a result of pathogen ascent starting as urethritis.[34] This hypothesis was confirmed by many studies simultaneously investigating pathogens isolated from the urethra or urine, as well as in epididymal aspirates or tissue,[27,35–40] in which an 84% identity of pathogens was demonstrated. Further, the bacterial ascent model was underlined by studies reporting an involvement of the prostate or seminal vesicles by biopsy, ultrasound or measuring prostate-specific antigen (PSA) changes.[29,33,37,41]

The pathogenic spectrum is related to the depth of microbiological investigations performed, as well as the study population investigated. In this context, etiological studies conducted before 1975 contain a diagnostic gap, specifically in young sexually active patients, since *Chlamydia trachomatis* as a relevant cause was by then unknown.[42] After the inclusion of *Chlamydia trachomatis* as a causative pathogen, many studies followed, investigating both sexually transmitted infections (STIs, e.g., *Chlamydia trachomatis*, *Neisseria gonorrhoeae*) and common enteric pathogens (e.g., *Escherichia coli*, *Pseudomonas* spp.). A combined analysis performed by our group included 758 patients from 14 studies and revealed detection of pathogens in 69.8% of cases. This could be increased to 87% with modern microbiological methods including pathogen culture and PCR analysis.[33] **Table 2** summarizes the pathogens that are frequently, occasionally or only rarely involved.

In a milestone study based on 24 patients published in 1977, epididymitis in patients under the age of 35 years were usually attributed to STIs, while in men above 35 years common uropathogens were mostly causative.[43] Unfortunately, this cut-off is still present in international guidelines,[44,45] whereas recent studies clearly provide evidence that STIs are not restricted to a specific age.[33,46]

Table 1: Possible etiologies of acute human epididymitis

Etiology	*Main cause*
Bacterial ascent	Common uropathogens
	Sexual transmitted infections
Viral genesis	*Mumps virus*
	Adenovirus
	Enterovirus
Fungi	*Candida albicans*
	Histoplasma capsulatum
Parasites	*Trichomonas vaginalis*
	Schistosoma spp.
	Filariasis
Drug-induced	Amiodaron
Rheumatic	Morbus Behcet
	Vasculitis
	Henoch-Schoenlein purpura
Obstruction	Vasectomy
Genital trauma	
Sterile reflux	
Idiopathic	

Table 2: Bacterial pathogen spectrum of acute human epididymitis

Frequent	Seldom	Rare
C. trachomatis	*Aerobacter* spp.	*Brucella* spp.
E. coli	*Enterobacter* spp.	*Corynebacterium* spp.
Klebsiella spp.	*Enterococcus* spp.	*Gardnerella vaginalis*
Neisseria gonorrhoeae	*Haemophilus influenzae*	*Salmonella* spp.
Proteus spp.	*Mycoplasma* spp.	*Staphylococcus epidermidis*
Pseudomonas aeruginosa	*Serratia* spp.	*Streptococcus pneumoniae*
	Staphylococcus aureus	
	Streptococcus spp.	
	Ureaplasma urealyticum	

E. coli: Escherichia coli; *C. trachomatis: Chlamydia trachomatis*

In addition to that of bacteria, a systemic spread of viral pathogens appears a plausible cause of epididymitis, yet studies on viral pathogens are scarce and generally indicate that *Mumps virus* and *Enterovirus* epididymitis represent rare causative entities.[33,47]

Symptoms

The leading symptoms are typically unilateral scrotal pain and epididymal swelling.[29,36,40] Bilateral involvement is restricted to only 4% of cases.[28,31,46,48–50] It takes on average 2–4 days from the onset of symptoms to medical consultation.[27,29,34,40,51,52] In most cases, infection affects the cauda epididymidis first before it ascends and reaches the testis in up to 90% of patients to inflict "epididymo-orchitis."[34,39,41,53] The clinical spectrum ranges from mild epididymal tenderness to severe systemic illness.[29,34,54,55] Signs of systemic infection (e.g., fever and shivering) are possible and are reported in up to 75%.[29,40,41,51]

Only about 30% of patients notice concomitant dysuria,[29,31,40,51] while the presence of typical urethritis symptoms is much lower and largely depends on the study population, with prevalence rates from 0% to 73%.[29,31,32,39,43,49,51,56] Of note, *Chlamydia trachomatis* is frequently present even in those cases without urethral symptoms.[33,35]

Diagnosis

Although sexual history is essential to help identify patients suffering from STIs, the sexual history was taken only in about 50% of consultations.[57,58] On the other hand, up to 50% of all STIs were detected in patients without a predisposing sexual history, underlining the need to screen all sexually active patients for STIs.[33]

Acute epididymitis is a clinical diagnosis with patients typically complaining of an enlarged and painful epididymis.[29,33,46] In young patients, it is essential to rule out testicular torsion. While scrotal ultrasound is not suggested in cases with simple epididymal enlargement,[44,46] it is beneficial for the assessment of severe cases, including abscess formation, and secondary testicular infarctions, as well as those cases with large reactive hydroceles hindering adequate palpation.[44] If a conservative therapy is chosen in severe cases, serial investigations are recommended.[54]

Laboratory investigations usually rely on white blood cell count and C-reactive protein assessment for disease confirmation and monitoring.[33,59] Unfortunately, a specific serum or seminal plasma marker for epididymitis is not available.

Since acute epididymitis usually results from the bacterial ascent, the identification of pathogens in the urine plays a key role in diagnosis. Urinalysis can be helpful since in about 80% of patients defining leukocyte counts can be found.[29,46,48] The current CDC and EAU guidelines recommend testing for STIs and culturing common urinary pathogens. According to the local facilities, urethral smears, as well as first void urine, are accepted when using either a Gram-stain or PCR analysis for STIs.[44,60] Despite these clear guidelines, up to 50% of patients receive an inadequate diagnostic workup.[58]

Currently, it is unclear if a further diagnostic advantage is possible by investigating prostate secretions or urine after prostatic massage.[61] On the other hand, microbiological diagnostics on semen samples at the acute infection stage is not recommended, because of pain and the low additional benefit.[49,50] Invasive procedures (e.g., epididymal aspiration) are obsolete because of the risk of obstruction.

It is of utmost importance to perform the microbiological investigations before starting antimicrobial therapy since afterward a bacterial pathogen can only seldom be detected.[33,50,52,58]

Management

Empirical antimicrobial therapy is of utmost importance and has to be chosen upon consideration of the most probable pathogens. In animal models, both tetracycline and fluoroquinolones have been demonstrated to have an excellent tissue penetration into the epididymis.[62–64] Unfortunately, only very few clinical studies on antimicrobial therapy are available. Of the different substances, tetracyclines have demonstrated good results in patients with suspected *Chlamydia trachomatis* involvement.[27,65] A breakthrough was the availability of fluoroquinolones in the 1980s, because of their efficiency against both *Chlamydia trachomatis* and common urinary pathogens.[38,66] The only randomized controlled trial confirmed the superiority of ciprofloxacin over pivampicillin and reported 20% versus 40% therapy failure rates, respectively.[46] These data are the basis of the CDC and EAU guidelines' recommending fluoroquinolones with activity against *Chlamydia trachomatis* as first choice, except in cases with *Neisseria gonorrhoeae*.[44,60] Unfortunately, in up to 50% of cases young patients received antimicrobial therapy that was ineffective against *Chlamydia trachomatis*.[30,57,58] Despite antibiotic resistance rates increasing worldwide in the past few years,[67] a recent study has shown that >85% of bacterial strains are still susceptible to both fluoroquinolones and third-generation cephalosporins, and thus confirmed the efficacy of current guideline recommendations.[33] However, no evidence-based recommendations can be given for how long the antimicrobial therapy should be given.

Hospitalization will be limited to patients with severe pain, high fever, or when patients are noncompliant.[44] Re-evaluation is suggested if symptoms do not subside within 3 days. In patients with confirmed STIs, the therapy of sexual partners is mandatory to prevent re-infection and spread of STIs.[49,51]

As an adjunct to therapy, bed rest, scrotal elevation, and analgesics are historically recommended. However, randomized studies are not available on these aspects. Surgical therapy is only rarely necessary and should be limited to patients with refractory epididymitis and those with secondary testicular infarctions. Of note, epididymal abscess formation – a classical indication for surgery – has been shown in a recent large study to resolve completely under conservative therapy.[33]

Clinical course

Conservative therapy is possible in the majority of patients, with only a few patients suffering secondary testicular infarction or persistent epididymal abscess formation requiring surgery.[33] Currently, about 80% of patients will be devoid of symptoms within 5–7 days,[52] with only 10% of patients continuing to report local pain after 30 days.[46] In contrast to the rapid reduction in symptoms, 40%–80% of men suffering epididymitis still have a palpable epididymal enlargement after 30 days, which persists in 20% of patients after 3 months.[46,66] Whether transformation into a chronic epididymitis is possible in such cases can only be hypothesized.[68]

Table 3: Animal models of experimental bacterial epididymitis

Infecting agent	Animal	Injection site	Bacteria count	Control	Duration of infection	Recovery of bacteria	Reference
E. coli	Rat	Cauda epididymidis, unilateral	10^6, 10^5, 10^4, 10^3 CFU in 200 µL	Saline, culture medium, or dead bacteria	1 day-7 days, 2 weeks, 3 weeks, 4 weeks; Biweekly up to 4.5 months	Not assessed	Lucchetta et al. 1983[81]
E. coli (O: 6)	Rat	Vas deferens, unilateral	10^6 CFU	Saline, antibiotic treatment	7 days before treatment	Yes	Nielsen, 1987[64]
E. coli (018 ab, ac)	Rabbit	Vas deferens, unilateral	10^6 CFU in 100 µL	Saline + tryptic soy broth	24 h, 48 h, 72 h, 1-week, 2 weeks, 1-month, 2 months, 4 months, 5 months	Yes, up to 2 weeks, not after 1-month	Hackett et al. 1988[74]
E. coli (E-19)	Rat	Vas deferens, unilateral	10^8 CFU in 100 µL	Tryptic soy broth or no injection	6 h, 12 h, 24 h, 48 h, 72 h	Not assessed	Tanaka et al. 1995[90]
E. coli (O: 6)	Rat	Vas deferens, unilateral	10^5 CFU in 100 µL	Antibiotic treatment (sparfloxacin)	1-24 h, 8 days, 14 days, 3 months, 6 months	Yes, up to 3 months	Vieler et al. 1993,[80] Ludwig et al. 1997;[62] Ludwig et al. 2002,[87] Pilatz et al. 2015[33]
E. coli (O: 6)	Rat	Vas deferens, unilateral	10^5 CFU in 100 µL	Saline	24 h	Yes, up to 24 h	Kaya et al. 2006[86]
E. coli (25922)	Rat	Vas deferens, unilateral	10^6 CFU in 100 µL	No injection or antibiotic treatment (ciprofloxacin)	12 days	Not assessed	Demir et al. 2007[88]
E. coli (CFT073)	Rat	Vas deferens, bilateral	4×10^6 CFU in 100 µL	Saline	7 days	Yes	Bhushan et al. 2008, 2011; Luu et al. 2013[84]
E. coli (25922)	Rat	Vas deferens, unilateral	10^5 CFU in 100 µL	Tryptic culture broth	3 days	Not assessed	Turner et al. 2011[91]
E. coli (DH5α)	Mouse	Epididymis, bilateral	7.5 µL of 2×10^6 or 2×10^7 CFU	Saline	3 days	Yes	Fei et al. 2012[76]
E. coli	Mouse	Vas deferens, bilateral	4×10^4 CFU in 5 µL	Saline	3 days, 7 days	Yes	Lang et al. 2013,[54] 2014[92]
E. coli	Mouse	Cauda epididymidis, bilateral	4×10^4 CFU in 50 µL	Saline	6 days	Not assessed	Cao et al. 2014[95]
E. coli (MTCC 729)	Rat	Vas deferens, bilateral	10^5 CFU in 50 µL	Saline	7 days	Yes, at all-time points	Biswas et al. 2015[83]
C. trachomatis	Monkey	Vas deferens, unilateral					Møller and Märdh, 1980[85]
C. trachomatis	Mouse	Cauda or caput epididymidis?	2.8×10^8 IFU in 40 ml	HeLa cells	3 days, 5 days, 7 days, 14 days, 21 days	Yes, up to 7 days, some tissues negative at 10-21 days	Kuzan et al. 1989
C. trachomatis (VR-123)	Rat	Vas deferens, unilateral	4×10^7 IFU in 40 ml	BGM cells	3 days, 7 days, 14 days, 30 days, 90 days	Yes, up to 90 days	Jantos et al. 1989,[78] 1992[77]
LPS	Rat	Intravenously	NA	Saline	0.5 h, 1 h, 2 h, 3 h, 6 h, 9 h, 15 h, 24 h	NA	Rodrigues et al. 2008[24]
LPS	Rat	Caput epididymidis, unilateral	NA	Saline	0.5 days, 1-day, 2 days, 3 days, 4 days, 5 days	NA	Cao et al. 2010[82]
LPS	Rat	Intraperitoneal	NA	Saline	3 h, 6 h, 9 h, 12 h, 15 h, 24 h	NA	Biswas et al. 2010

E. coli: Escherichia coli; C. trachomatis: Chlamydia trachomatis; LPS: lipopolysaccharide; NA: not available; CFU: colony-forming unit

Of much greater impact are recurrences, which are reported in up to 20% of cases and depend on the follow-up interval.[28,69] An analysis of insurance data demonstrated an increased risk for the development of a further recurrence with increasing numbers of previous recurrences.[70] This explains why surgery improving micturition (e.g., transurethral resection of the prostate) was performed in up to 27% of cases in two other studies.[28]

Impact on fertility

Despite epididymitis occurring frequently in patients within their reproductive years,[33] the impact on fertility has not been systematically investigated.[71] In the acute phase of the disease, leukocytospermia commonly reflects local inflammation of the urogenital tract.[50,72,73] With serial investigations, studies have shown reduced sperm concentrations following acute infection, with overall recovery within 3 months postantibiotic treatment.[52,66] However, persistent oligozoospermia and azoospermia, even after successful treatment, has been reported in up to 40% of patients.[71] The morphological reasons for this persistence are not clear and may either be related to a testicular dysfunction or an epididymal obstruction. Since a spread of the infection from the epididymis to the testis is frequently diagnosed by palpation or ultrasound, it is plausible that testicular inflammation contributes to these findings. Along this line, some older studies involving testicular biopsies taken at the acute phase described testicular inflammation

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characterized by polymorphonuclear neutrophils, macrophages, and interstitial edema.[39,41,52,72] Only one follow-up study, investigating two patients with azoospermia, showed testicular atrophy and bilateral testicular inflammation consisting of lymphocytic infiltrates, interstitial fibrosis, and largely reduced spermatogenesis 12 months after the acute infection. This was associated with a significant increase in FSH levels.[52] In contrast to these cases, a recent ultrasound study investigating 80 patients with unilateral epididymitis did not detect a testicular shrinkage compared with the healthy contralateral side 3 months after therapy.[54] This opens the possibility that epididymal duct obstruction plays a major role in the persistent low sperm count. This idea is supported by three patients in one study having azoospermia 3 months following epididymitis in conjunction with completely normal FSH values.[66] In summary, persistent azoospermia following acute epididymitis is not uncommon, with the exact patho-mechanisms still elusive. In this context, animal models mimicking the human situation are of considerable value.

ANIMAL MODELS OF EPIDIDYMITIS

Induction of bacterial epididymitis in animals
Animal models of bacterial epididymitis have been established in order to characterize the morphological changes and molecular pathways involved in the pathogenesis of epididymal infections, and to assess the efficacy of antimicrobial agents (**Table 3**). In rabbits,[74] mice,[54,75,76] and rats,[64,77–80] bacterial epididymitis is usually induced by uni- or bi-lateral inoculation of bacteria. Primarily *E. coli* and *Chlamydia trachomatis*, as the most clinically relevant pathogens, are injected into the lumen of the vas deferens to simulate the clinical ascending route of retrograde infection. In other models, bacteria are directly injected into the epididymal tissue.[81] Although other pathovars (*Staphylococcus aureus*, *Streptococcus veridans*) have also been evaluated for their ability to induce epididymitis in the rat,[81] *E. coli* remains most commonly employed and has subsequently been identified as most pathogenic for the epididymis and testis. An alternative tool to induce epididymitis is the administration of bacterial lipopolysaccharide (LPS), a major structural component of the outer membrane of Gram-negative bacteria, instead of live bacteria.[24,82,83] LPS is a well-characterized inducer of inflammation. It binds preferentially to TLR4 and induces a signaling cascade involving the accessory receptor proteins, MD2 and CD14, and several adapter proteins, such as myeloid differentiation factor 88 (MyD88). Finally, the transcription factor nuclear factor kappa B (NFκB) is activated, thereby triggering an innate immune response that resembles an infection with Gram-negative bacteria. LPS has been administered intravenously, intraperitoneally, or by direct injection into the organ to elicit epididymitis. The most commonly used model of bacterial epididymitis to date is the unilateral injection of *E. coli* into the vas deferens, with the rat as the predominant experimental animal. These models more recently have been transferred to the mouse in order to benefit from available transgenic animals. The mouse also serves as a model organism for noninfectious autoimmune epididymitis studies. This model was employed mostly to study detrimental effects of auto-antibody formation and the immunoregulatory role of regulatory T cells.[84]

Typical pathologies in experimental epididymitis
In accordance with clinical observations in epididymitis patients, reddening, swelling, and enlargement of the epididymis is one of the prominent signs of bacterial epididymitis in experimental animals.[54,62,74,75,77,83,85] Indications for the infection in the scrotum range from mild edema to severe erythema.[62,86,87] In the testis, atrophy, swelling, edema, and enlargement of the gonad are observed.[77,83,85] In contrast to men, in the animals models testicular weight was unaltered in the initial stages of the disease,[77,86] while a slight decrease in testicular volume was documented at later time points.[88] These changes reflect an acute response to the infection that appears within 24–48 h after inoculation,[54,86–88] and are most prominent up to 7–14 days postinfection, then subside and vanish after 1 month.[74,77,87] These features are in most cases confined to the infected side in unilateral injection models, and only reach the contralateral side in a few individual cases.[62,74,87,88]

Epididymal histopathology and inflammation
Direct injection of *E. coli* into the cauda epididymidis of rats induces local lesions in the cauda and causes inflammation that spreads to the testis in some animals.[81] In this model, no leukocytic infiltration is observed, but multi-nucleated cells and desquamated germ cells are detected in the lumen in the initial stages of the disease. This model is regenerative as the germ cell desquamation declines 8 days postinfection, and consistent germ cell numbers in the seminiferous epithelium are mostly retained several months after the infection. Observed effects are found to be independent of the number of bacteria injected. Noninflammatory lesions are also present in the testis in 40% of cases following injection of dead bacteria.

The induction of epididymitis by injection of *E. coli* into the lumen of the vas deferens in rats results in prominent morphological changes in the epididymis.[33,62,79,80,87,89,90] Purulent epididymitis is obvious from the infiltration of immune cells that is first visible at 24 h postinfection and becomes very prominent at 72 h.[90] Leukocytic invasion is often confined to the interstitium, but can also be observed intratubularly in some cases.[62,86,87,90] These cells are primarily lymphocytes and polymorphonuclear neutrophils.[62,86,91] Abscess formation and granulation of the tissue is evident, with the granules consisting primarily of neutrophilic granulocytes and macrophages.[80,86,87]

Similar observations have been made in rabbits after unilateral intraductal *E. coli* injection[74] with analysis of epididymides and testes several days, weeks, and months after the initial infection. An acute inflammatory response was evident by immune cell infiltration and the presence of neutrophils in both the vas and the epididymis, the development of sperm granulomas in chronic cases, and dilation of seminiferous tubules. Spermatogenesis was decreased in 10 out of 18 infected animals, with anti-sperm antibodies appearing 1 week after infection.

A mouse model of bilateral *E. coli*-induced epididymitis has been developed in our laboratory,[54,92] based on the previously described rat model of intraluminal injection.[79,89] Epididymal tissues in this model are characterized by immune cell infiltration (polymorphonuclear neutrophils, lymphocytes, and macrophages), epithelial damage, as well as interstitial edema and fibrosis (**Figure 1**). Inoculation of the epididymis with *Chlamydia trachomatis* induces similar histopathological alterations.[75,77,78,85]

As in men, epididymitis in animals also affects the testis to elicit a combined epididymo-orchitis. In the gonads of rabbits with epididymitis, dilation of the seminiferous tubules and the presence of multi-nucleated cells were observed.[74] In the rat models, testicular histology varies from no macroscopically evident signs of pathology[86] to lesions characterized by testicular inflammation with leukocyte infiltration, loss of germ cells, degeneration of epithelial cells, tubular atrophy, and interstitial fibrosis; this variability is likely due to the bacterial strains used and time points investigated.[77,78,81,88]

In all animal models with unilateral injection, the histopathological observations are confined to the infected side, with no sympathetic reaction of the contralateral testis evident.
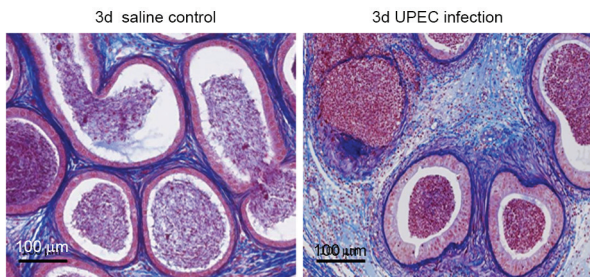
Figure 1: Murine cauda epididymidal histology 3 days after intraductal injection of uropathogenic *E. coli*. Pathological changes were observed as infiltration of immune cells, accumulation of collagen fibers, and loss of the epithelial cells. Azan staining was performed on fixed sections of 5 μm thickness.

Innate immune response in epididymitis

The innate immune response presents an important rapid defense mechanism against pathogens, and is characterized by infiltration of immune cells (dendritic cells, mast cells, monocytes/macrophages, natural killer cells, polymorphonuclear neutrophils, and expression of anti-infective peptides) (e.g., defensins) and increased pattern recognition receptor (e.g., TLRs, nucleotide-binding oligomerization domain 2 [NOD2]) expression and activation by the epithelium.

TLR4, in particular, is essential for the response of mammalian cells to LPS.[93] Upon activation of TLR4, a downstream signaling cascade involving MyD88 activation and NFκB binding ultimately results in expression of pro-inflammatory mediators and anti-bacterial peptides. Therefore, the assessment of the innate immune response in infection models - in addition to the histopathological analysis of immune cell infiltration described above - involves determination of an altered expression of TLRs, MyD88, defensins and cytokines, as well as increased NFκB-DNA binding activity. While no consistent change in TLR4 mRNA or protein level is detected in LPS-stimulated epididymal tissue, NFκB expression, and DNA binding activity are increased.[24]

Defensins are expressed throughout the epididymis with a characteristic segment-specific expression pattern, and exhibit a distinct, fine-tuned response to pathogen challenge. In LPS-induced epididymitis, defensin b2 (*Defb2*), *Defb21,* and *Defb27* mRNA expression decreased in the caput epididymidis,[82,94] while the expression of other defensins such as *Defb29*, *Defb41* and *Defb42* was unaffected by the treatment.[82] In turn, intravenous administration of *Defb21* to *E. coli*-infected rats significantly decreased the number of bacterial colony-forming units.[83] Sperm-associated antigen 11 (SPAG 11) is a peptide of the defensin family that is involved in sperm maturation and host defense mechanisms in the epididymis.[82] *Spag11b* (also known as *Bin1b*) expression decreases following bacterial infection in the murine epididymis.[54] In turn, overexpression of *Spag11b/Bin1b* renders mice more resistant to *E. coli*-induced epididymitis.[76] Considering the antimicrobial properties of these peptides and the limited information available on the effect of bacterial infection on their synthesis by the epididymal epithelium, a closer investigation of the segment-specific expression patterns of defensins in response to epididymitis is warranted.

The prominent immune cell infiltration observed in histopathological analyses of epididymitis (**Figure 1**) is accompanied by an increase in the mRNA expression of the pro-inflammatory cytokines interleukin (*Il*) *1b*, *Il6*, *Il12* and tumor necrosis factor (*Tnf*), and in the protein levels of IL1α, IL1β, and IL4.[91] TNF and IL6 protein levels either show a nonsignificant increase[91] or no change,[92] and interferon (IFN)-γ does not increase at the mRNA or protein level.[90,91,95]

As IL1 and TNF normally act synergistically in their response to inflammation, the insignificant elevation of TNF levels in epididymitis animal models, despite an increase in both IL1α and IL1β, could be explained by the time points selected that may not reflect the peak expression of this early response cytokine during the inflammation. Alternatively, the blunted increase in both TNF and IFN-γ levels seen in the murine model could indicate the ability of bacteria to promote an immunosuppressive environment.[92]

Obstruction and fibrosis

Long-term problems resulting from epididymitis in patients are very likely linked to the irreversible development of ductal obstruction and fibrotic tissue remodeling that occurs despite antibiotic treatment and eradication of bacteria. This highlights the importance of adjuvant therapies, which prevent the transformation of the tissue architecture and preserve fertility. Observation of the tissue morphology following *E. coli* and *Chlamydia trachomatis* inoculation in animal models reveals an epididymal fibrosis most prominently in the cauda epididymidis. It is characterized by the accumulation of fibroblasts and fibrocytes in the interstitium, the prominent formation of collagen fibers between the ducts, and flattening and destruction of the ductal epithelium[75,77,80,85,87] (**Figure 1**). This fibrotic transformation, accompanied by a loss of tissue architecture, is also evident in the testes in some models,[33,88] but not in others.[86] In our murine epididymitis model, no obvious fibrotic transformation is visible in the testis after 7 days of infection (unpublished data). Nonetheless, a significant correlation between the presence of bacteria and fibrosis in the epididymis is demonstrable,[80] but only a few experimental animal studies have assessed directly whether bacteria actually reach the testis.

The mechanisms underlying the fibrotic transformation in the epididymis are not well-characterized, and investigations in human patients are hindered by the lack of tissue samples for detailed analysis, mostly because biopsy collection is contraindicated in infected tissues. Nevertheless, pro-fibrotic factors, such as TGFβ and activin A, are expressed in the epididymis and follow - though inversely - the proximal-to-distal expression gradient.[18] Their role in fibrotic tissue remodeling in epididymitis warrants further analysis.

Effect of bacteria on sperm quality

The impairment of spermatogenesis or sperm maturation in animals with epididymitis is commonly observed histologically as desquamation of germ cells,[81] accumulation of sperm granulomas, and decreasing presence or complete absence of spermatozoa in the lumen of the epididymis or the seminiferous tubules.[74,88] In a limited number of epididymitis animal studies, sperm counts and the acrosome reaction were qualitatively and quantitatively assessed in more detail. Epididymal infection in rats induced a significant decrease in epididymal sperm number.[88] In our murine epididymitis *E. coli* infection model, a premature acrosome reaction and concomitant sperm nDNA fragmentation was apparent 3 days postinfection.[54] Clinically, the reason for oligozoospermia in approximately 30%–40% of epididymitis patients has not been conclusively identified.[71] It seems apparent from the observations in animal models that reduced sperm counts could be due to obstruction in the epididymis, impaired spermatogenesis, damage of spermatozoa by bacterial virulence factors, or a combination of all of these factors.

In vitro studies have been designed to elucidate the impact of bacterial presence on sperm quality. Direct incubation of human sperm cells with hemolytic *E. coli* strains diminished their motility and mitochondrial membrane potential and increased reactive oxygen

species, indicating the induction of apoptosis.[96] Furthermore, bacterial LPS has been shown to induce apoptosis in human spermatozoa directly.[97] This effect is mediated through the TLRs and causes damage to sperm mitochondria and the sperm plasma membrane.[98,99] In addition, sperm quality may be affected by the infiltration into the semen of leukocytes, which potentially can diminish motility, damage DNA and decrease sperm counts without direct involvement of bacterial products.[100] The correlation between the presence of leukocytes and bacteria with sperm quality has remained inconclusive to date.[100,101] In summary, analysis of tissue morphology in experimental epididymitis animal models has clearly demonstrated an impairment of sperm function following inoculation with bacteria, yet the cause of this damage and the mechanisms remain to be delineated. Similarly, the challenge also remains to differentiate precisely between damage caused by the inflammatory response of the host and the products of the invading pathogens.

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The authors declare that no competing financial interest exists.

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