Chapter 19
What is the impact of the immune system on male reproductive function?

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The immune system in the context of male reproductive biology

The primary role of the immune system is to protect its host from disease and facilitate repair following injury or organ damage. It does this via a complex network of leukocytes, secretions and receptors that support active immunity. Immunity is based on the recognition of molecules specifically expressed by pathogens or produced only during tissue damage (innate immunity), or through encountering structural motifs (antigens) that are not normally expressed during normal physiological functions, and are therefore considered to be "foreign" and potentially dangerous (adaptive immunity). This ability to discriminate foreign from "self" antigens, called immunological tolerance, is principally established during fetal and perinatal life. Consequently, spermatogenesis presents a unique problem for immunity, in that many molecules involved in spermatogenesis are not present during early development, and only arise during sexual maturation. As a result, the male tract and sperm especially are susceptible to damage by the immune system.

Crucially, the male reproductive organs are open to the external environment via the urogenital tract. Consequently, ascending infections, usually bacterial in nature, travelling through the urethra and up to the accessory glands, epididymis and testis, are common. These can be sexually transmitted (*e.g. Chlamydia trachomatis*, *Neisseria gonorrhoeae*), but the majority of such infections are due to commensal organisms (*e.g. Escherichia coli*, *Ureaplasma urealyticum*). The testis is also particularly susceptible to a number of sexually transmitted and even systemic viral infections, including mumps, human immunodeficiency virus (HIV), hepatitis B, and many emerging viruses, such as *Zika*. These infections commonly cause pain, cell and tissue damage and may lead to infertility.

The principal cell types responsible for immunity are the mononuclear phagocytes (macrophages and dendritic cells), lymphocytes (T cells, B cells and natural killer cells) and polymorphonuclear cells, such as neutrophils (Fig. 1). Fundamentally, the immune system is triggered when the mononuclear phagocytes respond to a perceived threat, either a known pathogen (via molecular pattern recognition receptors) or upon encountering a foreign antigen (via antigen-specific T cell and B cell receptors). The mononuclear phagocytes and neutrophils become activated very rapidly, secreting cytokines, complement factors and other molecules that induce inflammation (innate immunity). Inflammation is characterised by increased blood flow to the site of infection or injury, the recruitment of immune cells from the blood into the tissue and the production of cytotoxic substances and pain. This leads to tissue damage, which needs to be repaired during the resolution phase, once the immunological stimulus has been cleared. During inflammation, antigen-specific T cells are recruited and interact with the macrophages and dendritic cells, in a process called antigen-presentation, principally in the lymph nodes or other secondary immune tissue, such as the spleen. The activated T cells then target infected cells for killing, or activate antibody-producing B cells. This second phase involving T and B cells (adaptive immunity) usually takes several days to become effective, unless the host has been exposed to the antigens before through prior infection or immunisation.

Central tolerance occurs mainly in the thymus during early development, via clonal deletion to eliminate self-reactive T cells and the differentiation of regulatory T cells (Treg cells). These processes may not succeed in controlling autoreactivity in all circumstances and self-reactive T cells may persist or become activated later in life, resulting in autoimmune disease. Therefore, peripheral tolerance processes are important, wherein self-reactive T cells become functionally unresponsive (anergy) or are deleted when self-antigens are encountered outside of the thymus, throughout life. This involves continuous low-dose exposure of the self-reactive cells to their antigen.

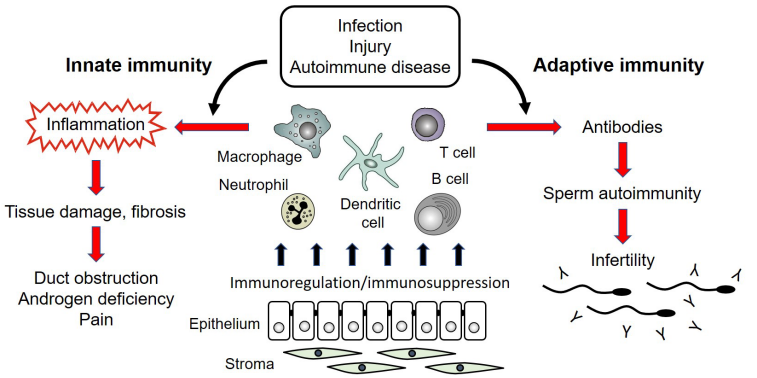

Figure 1: Diagram summarising the two arms of the immune system (innate and adaptive immune responses) activated by infection, injury or autoimmune disease in the male reproductive tract. Resident and circulating immune cells are normally under the regulatory influence of the epithelial and stromal cells, but immune activation leads to dysregulation of this control. This results in inflammation and damage to the cells and tissue of the male tract and the formation of sperm antibodies, causing temporary or chronic pain and infertility.

Immunoregulation and immune privilege in the testis

Spermatogenesis occurs within the seminiferous epithelium, which is avascular. All testicular blood vessels and lymphatics are located outside the seminiferous tubules in the interstitial tissue, and this is where the immune cells are also found. These include resident macrophage populations, which are found surrounding the tubules (peritubular macrophages) and throughout the interstitium (interstitial macrophages), as well as freely circulating T cells and NK cells. Immune cells are never observed within the normal seminiferous epithelium in the absence of inflammation or damage.

Most spermatogenic cells are immunogenic (i.e. not tolerised), and can be seen as foreign by the immune cells, so interaction between these cell types needs to be tightly regulated. The blood-testis barrier, more specifically the highly modified tight junctions between adjacent Sertoli cells, effectively isolates the later spermatocytes, spermatids and spermatozoa from immune cells and their secretions. However, the early spermatocytes and spermatogonia can also be immunogenic, and the blood-testis barrier is incomplete

or absent in the region of the rete testis. Moreover, experimental studies have shown that foreign grafts into the interstitial space, outside the blood-testis barrier, are also protected, an observation that was called "immune privilege". Consequently, active regulatory mechanisms are required to prevent immune activation and inflammatory responses from occurring: these are immunosuppression and Treg cell-mediated peripheral tolerance.

Immunosuppression involves locally produced factors that inhibit macrophage and lymphocyte activity, including androgens and other immunoregulatory lipids, lymphotoxic molecules, such as Fas ligand (CD95L), complement and protease inhibitors, and immunosuppressive cytokines, including interleukin-10, transforming growth factor-β and activin A. These factors are produced by the Sertoli cells, Leydig cells and resident macrophages. Sertoli cells and testicular macrophages are inherently immunosuppressive in vitro and can be used to support graft survival in transplantation experiments (Chapter 7). While spermatogenic cells are confined to the seminiferous epithelium, spermatogenic cell antigens constantly leak from the seminiferous tubules, where they are taken up by the resident macrophages, and presented to circulating Treg cells, thereby maintaining antigen-specific Treg-dependent physiological tolerance against the spermatogenic cells.

Immunoregulation in the efferent ducts, epididymis and vas deferens

The local immune environment in the post-testicular tract is quite different from that of the testis. Although the typical epithelial tight junctions are present, there is no complex structure analogous to the blood-testis barrier between Sertoli cells in the duct epithelium. Furthermore, evidence for extended graft survival is lacking. Most strikingly, numerous intraepithelial mononuclear phagocytes are found in the efferent ducts, initial segment and caput epididymidis. These cells appear to physically interact with the sperm exiting from the testis, and are believed to be important for maintaining peripheral tolerance towards the sperm. In the corpus and cauda of the epididymis, these intraepithelial cells are less prominent, and resident interstitial macrophages are more frequently observed. These differences in the distribution of immune cells, as well as differences in the vascularity of the regions, appear to be responsible for the very different responses of the caput and cauda regions of the epididymis to immune activation and inflammation

(Fig. 2). In experimental autoimmune and infection models in rodents, extensive inflammatory and fibrotic responses are largely confined to the cauda region, which can lead to permanent damage to the epididymal duct. By contrast, the caput epididymis is largely unaffected by these stimuli. These regional differences are associated with different sperm antibody responses in different regions of the male reproductive tract as well. Sperm antibody formation in men is more frequently associated with damage or congenital absence of the more distal regions of the epididymis and the vas deferens, in comparison with the more proximal regions of the epididymis.

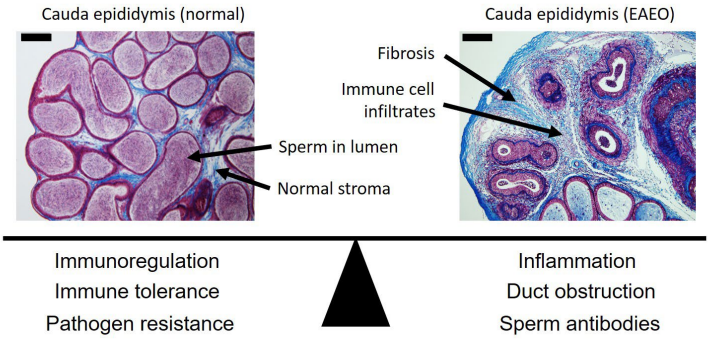

Figure 2: The balancing act of the interaction between the immune system and male reproduction. Under normal physiological conditions, immunoregulation and peripheral tolerance co-exist with endogenous pathogen resistance mechanisms. Left side: micrograph shows a normal mouse cauda epididymis with abundant sperm in the duct lumen and a relatively low proportion of stromal tissue. Right side: micrograph shows a mouse cauda epididymis damaged by experimental autoimmune epididymo-orchitis (EAEO), displaying few sperm, epithelial disruption, extensive fibrosis and immune cell infiltrates. Bar = 200 μm

Physiological role of the immune system in male reproduction

In addition to providing vital protection against reproductive tract infections, the immune system plays a key role in the development and ongoing function of the male reproductive tract. Macrophages

are important for the normal development of the testis, regulation of the spermatogonial niche, maturation and proliferation of the Leydig cells and even steroidogenesis in the adult testis. Genetic ablation of macrophages throughout the body results in male fertility defects in mice, highlighting the importance of these cells in normal reproductive function.

Paradoxically, inflammatory networks have been shown to play a role in the cycle of the seminiferous epithelium. Several inflammatory and immunoregulatory mediators produced by the Sertoli cells, including interleukin-1 and activin A, are involved in the regulation of spermatogonial differentiation and proliferation, meiotic progression and regulation of the blood-testis barrier during spermatocyte transition into the adluminal compartment. This appears to involve inflammatory responses of the Sertoli cells, potentially triggered by the residual cytoplasm from released spermatozoa.

Clinical issues and pathophysiology

While autoimmune disease is rare in the male reproductive organs, sperm autoimmunity can occur when mechanisms of self-tolerance are disrupted or deficient. Physical damage to the male reproductive tract that may occur due to blunt trauma, testicular biopsy, or vasectomy can lead to the formation of sperm antibodies. This results in sperm agglutination and inhibition of sperm motility, as well as blocking surface receptors crucial for fertilization of an oocyte. Immune system involvement in male infertility is highlighted by the fact that testicular biopsies from infertile men frequently contain immune cell infiltrates. T cells are commonly found in biopsies from men with hypospermatogenesis, and immune infiltrates, characterized by T cells, B cells and dendritic cells, are a predominant feature of testicular cancer. Although the clinical significance and consequences of autoimmune inflammation of the testis and epididymis in humans can be difficult to assess, experimental rodent models of autoimmune orchitis and epididymitis show that inflammatory and fibrotic damage in these organs is associated with sperm antibody formation and infertility.

Infections of the male reproductive tract and the resulting inflammation can severely impair fertility, causing damage and fibrosis, both of which can lead to loss of the seminiferous epithelium, and obstruction of the epididymal duct and vas. The steroidogenic Leydig cells are usually affected, resulting in reduced

testosterone levels. In addition, inflammation is also believed to be responsible for ageing/damaged Leydig cells and hypogonadism in older men. Animal studies provide evidentiary support for the use of concurrent anti-inflammatory therapy combined with anti-microbials in treating infectious diseases of the male reproductive system, in order to minimise damage arising from excessive inflammation.

The testis is a common target for viruses.. Many viruses, including mumps, herpes simplex virus-2, hepatitis B and HIV can damage reproductive function in the male. Moreover, viruses can remain sequestered in the testis, due to its immune-privileged environment. This can be a major public health concern, since certain emerging viruses, such as Zika, can be shed in semen of asymptomatic men for up to a year.

In contrast to the testis, the epididymis is primarily the target of infection for ascending bacterial pathogens. In fact, acute epididymitis is one of the most frequent medical presentations in the urological clinic, and is on the rise globally. Although empirical antimicrobial therapy eliminates the pathogen, up to 40% of these patients develop oligozoospermia or azoospermia. Animal studies have shown that the fibrotic damage and obstruction that develops following epididymitis is mostly a consequence of the host immune system response and uncontrolled inflammation.

Prostatitis is most commonly due to ascending urogenital infections, although transurethral manipulation procedures including urethral catheterization or transrectal prostate biopsy can also lead to inflammation of the prostate. While acute bacterial prostatitis responds promptly to antimicrobial therapy, chronic prostatitis may result in chronic pelvic pain. Urethritis is commonly caused by *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium* and Herpes simplex virus, all which are sexually-transmitted. If left untreated, urethritis can develop into ascending infections of the reproductive organs, affecting fertility.

Suggested reading

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