The Immune Privilege of the Testis

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2.1.1 Introduction

Male germ cells (GC) enter meiosis beginning their complex transition into highly specialized spermatozoa at the time of puberty, after the establishment of immune competence. During the process, a myriad of surface and intracellular proteins are expressed, yet these new autoantigens are tolerated by the testis. The immunogenicity of the proteins is not diminished, as shown by their ability to induce strong autoimmune reactions when injected elsewhere in the body [1, 2]; rather it is the testis itself that confers protection. Initial suggestions that the testis was an immune privileged site were substantiated experimentally when histoincompatible allo- and xenografts placed into the interstitial space of the rat testis survived and prospered for an indefinite period of time [3]. Similarly, ectopically transplanted allogenic Sertoli cells (SC) not only survive, but when co-transplanted with allogenic pancreatic islets, also resist rejection without additional systemic immunosuppression in animals [4]. More recently, the transplantation of spermatogonia in germ cell depleted testis could restore spermatogenesis even across species borders in some instances [5]. There is general agreement that immune privilege is an evolutionary adaptation to protect vulnerable tissues with limited capacity for regeneration, thereby avoiding loss of function [6, 7]. For the testis this means safeguarding reproductive capability. Notwithstanding its immune privileged status, the testis is clearly capable of mounting normal inflammatory responses, as proven by its effective response to viral and bacterial infection. In pathological circumstances, the misbalance between the tolerogenic and the efferent limbs of the testicular immune response can lead to the formation of autosperm antibodies and in rare instances, autoimmune epididymo-orchitis in humans. Immune infertility is now estimated to be a considerable cause of sterility in couples seeking medical assistance [8–12].

The most commonly used model for the investigation of autoimmune-based inflammatory testicular impairment is experimental autoimmune orchitis (EAO), a rodent model

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based on active immunization with testicular homogenate and adjuvants [13]. The clinical term "orchitis" is particularly attributed to acute, symptomatic disease due to local or systemic infection, whereas subacute or chronic, asymptomatic inflammation of the testis including noninfectious disease is difficult to diagnose and therefore likely to be ignored [14]. Orchitis may also occur in conjunction with infections of the genitourinary tract and as manifestation of sexually-transmitted diseases such as gonorrhea or *Chlamydia trachomatis* [15]. Urethral pathogens, i.e., *Escherichia coli* cause bacterial epididymo-orchitis [16]. The most common cause of viral orchitis is mumps. On balance, these data clearly indicate that the mechanism underlying immune privilege in the testis and its disruption by pathological alterations are matters of clinical importance and hence continued scientific interest. The following sections highlight some of the mechanisms that are associated with the establishment or maintenance of immune privilege.

2.1.2 Blood Testis Barrier

The blood testis barrier (BTB) is comprised of various integral membrane proteins, which in turn contain a number of interesting components such as junctional adhesion molecules (JAMs) and claudins 1 and 11 along with claudins 3–5, claudins 7–8 (also identified in the testis), and occludin [17]. The BTB divides the seminiferous epithelium into two distinct compartments: the basal carrying the spermatogonia, leptotene, and zygotene spermatocytes; and the adluminal with meiotic pachytene and secondary spermatocytes, haploid spermatids, and spermatozoa, which are all completely engulfed by cytoplasm protrusions of the Sertoli cells. The main task of the BTB is to protect the developing GC from the immune system. Meiotic and postmeiotic GC, including spermatozoa (daily production: 150×10^6 spermatozoa in rat [18]), express a large array of neo-antigens that first appear during puberty, long after the establishment of self-tolerance. With the instigation of spermatogenesis, the BTB is concurrently established and immediately sequests postpubertal GC from the immune system (Fig. 2.1.1).

Impairment of BTB integrity has been observed during inflammation, infection, and trauma which ultimately result in germ cell loss [19–21]. Mechanistically, elevated levels of tumor-necrosis factor (TNF)-α and transforming growth factor (TGF)-β, found in systemic and local testicular inflammation [22–25], have been shown to perturb the assembly of the tight junctions in cultured SC probably by downregulating occludin expression [26, 27]. Despite the junction's ability to isolate meiotic and postmeiotic GC from circulating antibodies and leukocytes, it is now accepted that the BTB alone does not account for all the manifestations of the testicular immune privilege. A proposition supported by the findings is that germ cell autoantigens are present in the basal compartment in spermatogonia and early spermatocytes which are not protected by the BTB [28, 29]. Moreover, the BTB is incomplete in the rete testis, a location where immense numbers of spermatozoa with newly adapted surface molecules traverse towards the epididymis, making it a particularly susceptible region for the development of autoimmune orchitis. Furthermore,

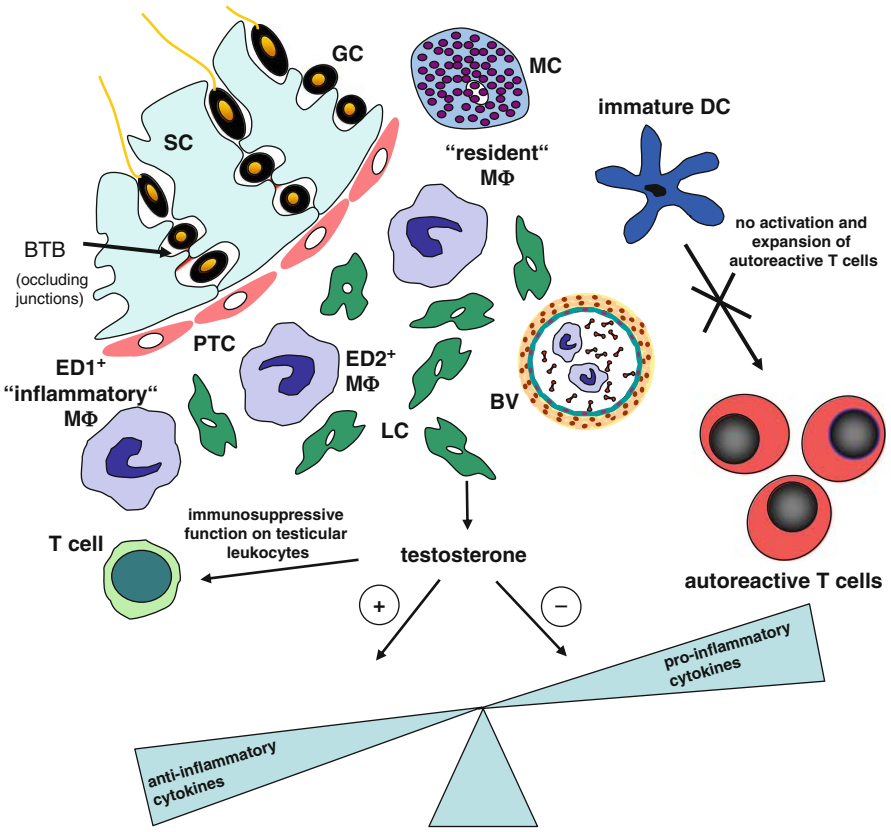

Fig. 2.1.1 Hypothetical model of factors maintaining the testicular immune privilege. The blood testis barrier (BTB) connects neighboring Sertoli cells (SC) and segregates the majority of neoantigen expressing meiotic and postmeiotic germ cells (GC) from the testicular immune system. In the interstitial space, the ED2+-resident type of macrophages (MΦ) with their immunoregulatory and trophic functions constitute the largest subpopulation of leukocytes, whereas the ED1+ "inflammatory" macrophage cohort is much smaller in number. Most likely the phenotype of testicular dendritic cells (DC) in normal testis inhibits an activation and expansion of autoreactive T lymphocyte clones. The concentration of testosterone in the testicular interstitial fluid synthesized by the Leydig cells (LC) is about 8–10 times higher than that in serum. Recent data point to an increasingly important immunosuppressive role of androgens in inhibiting leukocyte function and reducing proinflammatory cytokine expression. *BV* blood vessels; *PTC* peritubular cells; *MC* mast cells

Head and Billingham [30] showed extended survival (i.e., no immune response/attack) of allografts that were placed under the organ capsule in the testicular interstitium. Therefore, some other mechanism, besides physical separation, must exist to maintain testicular immune privilege, which requires more robust protection of the tolerogenic environment of the testis.

2.1.3 Endocrine Regulation of Testicular Function and Immune Privilege

In addition to the well known anabolic and spermatogenic effects, a role for androgens in down regulating proinflammatory cytokines has now been shown in both experimental and clinical studies. Incubation of stimulated human monocytes, macrophages, and several nonimmune cell types with testosterone, resulted in the suppression of adhesion molecules and cytokines such as IL-1, IL-6, and TNFα and increased production of anti-inflammatory cytokines such as IL-10 [31–34]. Testosterone is also involved in T cell apoptosis [35]. A direct connection between sex steroid levels and testicular immune privilege was shown by Head and Billingham [36], when in transplantation studies, rats pretreated with estrogen to suppress Leydig cell testosterone production, promptly rejected intratesticular allografts in direct contrast to the reaction of their untreated cohorts. These studies indicate that high local testosterone concentrations, characteristic for the testis, seem to play an important role in the maintenance of testicular immune privilege. However, the precise manner in which testosterone mediates its anti-inflammatory functions on testicular leukocytes is as yet unknown. What can be surmised from the available data is that it appears likely that androgens exert their immunosuppressive function on testicular leukocytes either via nongenomic pathways [37] or indirectly by regulating the balance between pro and anti-inflammatory cytokine expressions in the Sertoli, Leydig, and peritubular cells (PTC).

2.1.4 Mechanism of Maintenance and Disturbance of Testicular Immune Privilege

2.1.4.1 Macrophages

Under normal conditions macrophages and all other leukocytes are exclusively found in the interstitial space; in humans they are also found in the tubular wall, but never within the seminiferous epithelium. There is little doubt that macrophages play a central role in the establishment and maintenance of the immune privilege of the testis. This supposition was first substantiated by in vitro studies where testicular macrophages displayed a reduced capacity to synthesize IL-1β and TNFα compared with macrophages from other tissues [38–40], and exhibited immunosuppressive characteristics [39, 41]. In the rat testis, at least two subsets of macrophages can be discerned. This heterogeneity has functional implications as in the testis the ED1+ “inflammatory” subsets, but only few ED2+ resident macrophages, express MCP-1 and iNOS in untreated and LPS challenged rats [42, 43]. The ED2+ resident population of testicular macrophages does not participate in promoting inflammatory processes; it is thought to have an immunoregulatory role in maintaining immune privilege and tropic functions, particularly on Leydig cells. Clear evidence points out that the ED1+ED2- monocytes/macrophages are involved in the testicular inflammatory

response and it is the influx of ED1+ monocytes during acute and chronic inflammation that drastically alters the composition of the macrophage population and shifts the cytokine balance in favor of an inflammatory response with the potential to overcome the immune privilege [42–44].

2.1.4.2 Dendritic Cells (DC)

DC are a heterogeneous population that belongs to the most important antigen presenting cells (APC) and play a major role in the initiation and orchestration of primary immune responses of both helper and cytotoxic T and B lymphocytes – the effector cells of the adaptive immune system. DC not only activate lymphocytes, but also tolerize T cells to antigens, thereby minimizing autoaggressive immune responses [45]. DC migrate as immature or precursor cells from the bone marrow into peripheral tissue where, upon receiving an activatory signal associated with pathogens or inflammation, they migrate to the local lymph nodes, mature, and present the antigens to T cells captured in the periphery [45]. Immature DC have the highest capacity to internalize antigens, but have low T cell stimulatory activity, whereas mature DC downregulate their endocytic activity and are excellent T lymphocyte stimulators [46]. Mature DC are characterized by the upregulation of surface T cell co-stimulatory (CD40, CD80 and CD86) and MHC class II molecules, the production of bioactive IL-12 and TNFα, and changes in migratory behavior [47]. Both MHC class I and II expressions occur within the interstitial tissue of the testis including the macrophages and Leydig cells. Our own results show that also testicular DC express MHC II molecules. In contrast, on the developing GC MHC antigens are reduced in number or absent. These data indicate that spermatogenic cells are able to avoid direct recognition by CD4+ and CD8+ T cells, which may be important for reducing the potential for antigen specific immune response elicited by DC or macrophages in the seminiferous epithelium [48–53]. In spite of their potential importance in maintaining the balance of the testicular immune status between tolerance (immune privilege) and (auto-)immunogenic reply, DC in the male gonad have received little attention in the past. The presence of DC in normal (approximately 1×10^5 cells) and chronically inflamed testes from Wistar and Sprague-Dawley rats was determined and quantified for the first time using DC specific markers (Ox62 and CD11c) [54]. In experimentally induced autoimmune orchitis (EAO), DC were found in the interstitial space of the testis and, in large numbers, in the granulomas. Although increases of between 5.5- (CD11c) and 8-fold (Ox62) were seen compared to controls, these quantities are still significantly lower than the number of macrophages found in similar circumstances [54–57]. Testicular DC isolated from EAO animals significantly enhanced naïve T cell proliferation compared with control DC from untreated animals suggesting a more tolerogenic phenotype for DC in normal testis function, thereby maintaining immune privilege [58].

In light of the "danger model," the recent characterization of numerous heat shock proteins (e.g., Hsp60 and Hsp70 among others) as testicular autoantigens could provide a mechanism of how DC in the testis participate in the activation of autoreactive lymphocytes and in the subsequent damage of testicular tissue, thereby overcoming the immune

privilege [59]. Millar et al. [60] provided evidence that Hsp70, when released by necrotic cells, acts like a danger signal by enhancing the maturation of DC, which then trigger autoimmunity. It is important to note that the release of endogenous inflammatory signals (e.g., Hsp70) requires necrotic cell death such as that resulting from infection or injury. On the basis of our own data and that of other autoimmune disease models, we hypothesize that immature DC, normally involved in maintaining immune privilege, under inflammatory pathological conditions sense self antigens like Hsp70 as danger signals and after maturation may overcome immune privilege/immune tolerance by local activation and expansion of autoreactive T cells.

2.1.5 Conclusions

There is now widespread agreement that the immune system, spermatogenesis, and steroidogenesis, the intrinsic testicular functions, are intricately linked by a network of complex interactions. The importance of the delicate balance needed, between the suppression of the immune response to protect the GC from autoattack on the one hand and the ability to have an active immune response to prevent damage from infection, trauma, and cancer on the other, is reflected by the fact that in the human male about 12–13%, in some studies even more, of all diagnosed infertility is related to an immunological reason, while its contribution to idiopathic infertility (31% of all cases) remains unknown [8, 10–12]. The mechanisms responsible for the testes' immune privilege are still far from being understood, but it is apparent that the identified factors involved are multiple and probably redundant. Overall, long regarded as a peculiar side issue of testis function, immune privilege is now established as part of the general scheme of male gamete formation and successful reproduction. Further research in the area will not only help to improve diagnosis and treatment of immunological male infertility, but will also open new avenues in contraceptive development and transplantation medicine.

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