REVIEW

Testis Immune Privilege

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Abstract: There are several immune privileged sites including the testis, anterior chamber of the eye, brain, central nervous system, maternal fetal interface of the placenta, hair follicles and some tumors. The testicular environment is not always tolerogenic or immunologically ignorant. The local factors and cellular components of the testis determine the immune response. It is now known that immune cells (antigen presenting cells, T cells and NK cells) involved in mounting an effective immune response are present in the testis. Both endocrine and paracrine networks coordinate to regulate testicular immune privilege. Tregs contribute to testicular immune privilege. The role of androgens in testicular immune regulation has long been underestimated; yet, accumulating evidence now shows that they orchestrate the inhibition of proinflammatory cytokine expression and shift cytokine balance toward a tolerogenic environment. Androgens also regulate the testicular immunoprivileged status. The androgens synthesized by Leydig cells suppress both systemic and testicular immune responses to auto-antigens. Moreover, several negative regulatory immune systems have been found in the testis. In particular, numerous paracrine cytokines, including various anti-inflammatory factors, would contribute to the maintenance of testicular immune privilege. The testis represents a distinct immunoprivileged site where both allo-antigens and immunogenic auto-antigens can be tolerated without evoking detrimental immune responses. Testicular innate immunity is particularly critical when systemic immunity is reduced. Here, we discuss the local cellular innate immune defense system of the testis. Impairment of immune homeostasis in the testis can result in orchitis, an etiological factor of male infertility. This review will focus on immune privilege in the testis.

Keywords: *immune cells, immune privilege, immunological microenvironment, blood-testis barrier, innate immunity, testis.*

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Testicular immunology is the study of the immune system within the testis. It includes an investigation of the effects of infection, inflammation and immune factors on testicular function. Two unique characteristics of testicular immunology are evident: a) the testis is described as an immunologically privileged site, where suppression of immune responses occurs; and, b) some factors which normally lead to inflammation are present at high levels in the testis, where they regulate the development of sperm instead of promoting inflammation. The protection of germ cells from autoimmune attack, the "immune privilege" of the testis, was originally attributed both to the existence of the blood-testis barrier and

to a failure of the testicular immune system to respond to antigens [1].

The presence of immune privilege in the human testis is controversial and insufficient evidence exists to either confirm or rule out this phenomenon. The major functions of the testis are spermatogenesis and steroidogenesis. The mechanisms controlling immune privilege seem to involve factors that also control spermatogenesis and steroidogenesis. The testis is composed of various cell types including immune cells and testis-specific cells. The testis is a distinct immune privilege site. While the testis is a remarkable immune privilege site, it is well connected to afferent lymph nodes. Therefore, the testis has most types of immune cells, including macrophages, T lymphocytes, dendritic cells, and mast cells. Furthermore, the role of the testicular dendritic cells in suppressing antigen-specific immunity and T-lymphocyte activation is discussed. Macrophages are by far the most prevalent cell type in the testicular interstitium, in close morphological association and functional interaction with Leydig cells. Macrophages and dendritic cells belong to the heterogeneous group of cells collectively called “antigen-presenting cells. These immune cells are important in the maintenance of the special testicular immune environment. Although the testis is an immunoprivileged organ, immune cells present in the interstitium retain the ability to mount inflammatory and innate immune responses [2].

An immune privileged status does not indicate that the site has no effective immune response. Immune privilege represents a special microenvironment where the systemic immune responses to allo- and autoantigens are remarkably reduced. Sperm production and androgen synthesis are two key functions of the testis. Testicular immune privilege protects germ cells from immune attack under physiological conditions. The first wave of spermatogenesis occurs in puberty, a long time after immune competence is established during the fetal and neonatal period. Therefore, most germ cells are foreign to the immune system, and large numbers of germ cell antigens are immunogenic. These autoantigens are tolerated in the testis although they induce autoimmune responses in extratesticular sites [3].

This review article highlights the unique immune environment of the testis, particularly focuses on the regulation of testicular immune privilege.

Immune protection of the testicular functions is critical for the maintenance of reproduction and development of a species. The production, differentiation, and presence of male gametes represent inimitable challenges to the immune system [4].

How the testicular environment suppresses the immune response is only partially understood. The mechanisms underlying testicular immune privilege have been investigated for a long time. Increasing evidence shows that both a local immunosuppressive milieu and systemic immune tolerance are involved in maintaining testicular immune privilege status. While physical structures like the blood-testis barrier have been implicated, antigen sequestration, aberrant lymphatics, or impeded immune cell access is not the underlying cause of testicular immune privilege, and it is increasingly evident that privilege involves active immunoregulation and local immunosuppression. Effective regulation of autoimmune reactions against spermatogenic antigens and inflammatory immune responses against pathogens is critical to protect male fertility. Production of androgens (mainly testosterone) is a major function of Leydig cells. Androgens exhibit immunosuppressive activities that contribute to different immune responses between the sexes. More specifically, the unique somatic cells of the testis, the Sertoli cells of the seminiferous epithelium, and the steroidogenic Leydig cells, together with the large resident testicular macrophage

population, have been directly implicated in suppressing or regulating immune responses to antigens located within the testicular environment.

Androgens play roles in maintaining the balance between autoimmunity and tolerance.

The testis is a remarkable immune privilege site where immunogenic germ cells are protected from detrimental immune attack. The testis adopts effective local innate defense mechanisms against microbial infections. Breakdown of immune homeostasis in the testis may lead to orchitis and impair male fertility. Certain sites of the human body have immune privilege, meaning they are able to tolerate the introduction of antigens without eliciting an inflammatory immune response. Immunologically privileged sites include: the eyes, the placenta and fetus, the testicles, the central nervous system, the anagen hair follicles. Immune privilege is thought to be an evolutionary adaptation to protect vital structures from the potentially damaging effects of an inflammatory immune response. Antigens from immune privileged regions have been found to interact with T cells in an unusual way: inducing tolerance of normally rejected stimuli. Immune privilege has emerged as an active rather than a passive process. Physical structures surrounding privileged sites cause a lack of lymphatic drainage, limiting the immune system's ability to enter the site. Other factors that contribute to the maintenance of immune privilege include: low expression of classical MHC class Ia molecules; expression of immunoregulatory nonclassical, low polymorphic class Ib MHC molecules; increased expression of surface molecules that inhibit complement activation; local production of immunosuppressive cytokines such as TGF-β; presence of neuropeptides; constitutive expression of Fas ligand that controls the entry of Fas-expressing lymphoid cells.

Testicular immune privilege is an active phenomenon where immunomodulatory factors expressed or secreted locally by different testicular cells control the overall immune response. The testis is an organ that is highly specialized for the production of sperm and male sex hormones. The testis has long enjoyed a reputation as an immunologically privileged site based on its ability to protect auto-antigenic germ cells and provide an optimal environment for the extended survival of transplanted allo- or xeno-grafts [5].

The testis exhibits special defense mechanisms considering its remarkable immunoprivileged status and effective local innate immunity. The testis is a complex organ with a unique physical structure and a large number of cell types. The mammalian testis consists of two distinct compartments: the seminiferous tubules and the interstitial spaces between the tubules. Testicular immune privilege was initially proposed to be attributed to the absence of lymphatic drainage, which was challenged by the discovery of the afferent lymphatic vessels in the testis. The testis has effective local innate immunity against invading pathogens [6].

The testis is structurally complex regarding the blood-testis barrier and the many different cell types it contains. While the testis structure is not fully responsible for the immune privilege, it is involved partially in the maintenance of the special testicular immune environment [7,8].

Similar to the brain, which has the blood brain barrier, the testis has the blood-testis barrier [5]. The blood–testis barrier is likely to contribute to the survival of sperm. The Sertoli cells play a crucial role in the protection of sperm from the immune system. They create the Sertoli cell barrier, which complements the blood-testis barrier. The protection is ensured by tight junctions, which appear between two neighboring Sertoli cells. Another mechanism which is likely to protect sperm is the suppression of immune responses in the testis [9]. The sequestration of auto-antigens from the immune system by the blood-testis barrier was believed to be critical for testicular

immune privilege. However, the interstitial spaces and early-stage germ cells that localize outside the blood-testis barrier, including spermatogonia and preleptotene spermatocytes, also benefit from immune privilege. The testis can be infected by various microbial pathogens derived from circulating blood or that ascend the genitourinary tract. To elicit an appropriate and effective local response against invading pathogens, testicular cells have to overcome immune privilege [10].

The breakdown of local testicular immune homeostasis may lead to orchitis, an etiological factor of male infertility. Testicular defense mechanisms have two aspects: protection of auto-antigens from detrimental immune responses and counteraction of invading microbial pathogens. Numerous local immunoregulatory cytokines, including both pro-inflammatory and anti-inflammatory factors, are involved in the regulation of the testicular immune environment. Androgens would contribute to testicular immune privilege by negatively regulating the local immune responses and systemic tolerance to auto-antigens [6].

The mechanism(s) or factor(s) responsible for testis immune privilege are various components such as anatomy (lymphatic drainage, blood testis barrier; the blood-testis barrier B/Sertoli cells barrier), physiology (lower temperature of the scrotum, higher zinc concentration), immunology (macrophages, dendritic cells, T cells and NK cells), and testicular cells (Leydig cells, myoid cells, Sertoli cells and germ cells) [11].

The testis contains a high zinc concentration as compared to other organs, and therefore it was thought that high zinc levels or zinc dependent enzymes in the testis could be important in abolishing the immune reaction [5].

Collectively, these data suggest that testicular immune privilege is not permanent and the balance can be tipped, leading to autoimmune orchitis or destruction of the allo- or xeno-grafts transplanted in the testis [12].

The presence of immunosuppressive activity in testicular fluid has been evidenced by several studies. Testicular immune privilege is a complex phenomenon which is the result of the combined contribution of testis cellular components rather than a single cell type acting alone [13-15]. While the testis is a remarkable immune privileged site, chronic orchitis and autoimmunity are important etiological factors of male infertility.

The testis is specific in that it produces haploid germ cells of which autoantigens newly appear long after the neonatal immune tolerance. Under normal condition, these autoantigens are protected by the blood-testis barrier formed by Sertoli cells [16].

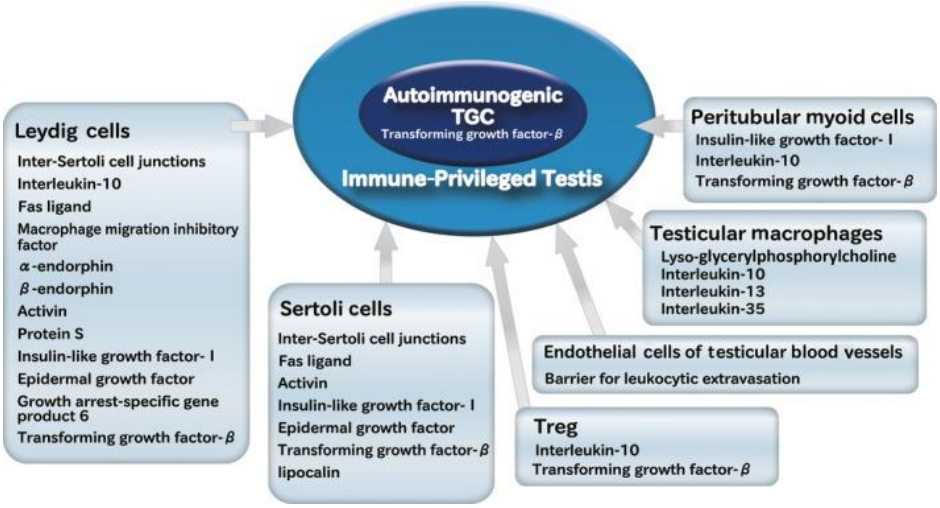

Fig. 1. Immunological microenvironmental in the testis [17].

Thus, the testis is an immunologically privileged site where haploid cells are protected from autoimmune attack. Not only the blood-testis barrier but also various immuno-suppressive factors are involved in the immune-privileged testis. Immune cells residing in the testicular interstitial space form the immunological microenvironment of the testis. They are assumed to play a role in maintaining testicular homeostasis and immune privilege [17]. Testicular immune homeostasis can be disrupted by various pathological factors, thereby resulting in inflammatory conditions that may impair testicular functions. To overcome this immunoprivileged status, testicular cells are equipped with innate defense machineries that function against invading microbes [18]. The maintenance of immune homeostasis is necessary for testicular function and male fertility. The innate immune response is the first line of the body's defense against invading microbes. While the testis is an immunoprivileged organ, it may be infected by various microorganisms via hematogenous dissemination and ascension of the genital tracts [19].

The mechanisms underlying this privilege remain poorly understood compared with more intensively studied models of immune privilege, such as the eye and feto-uterine unit, but evidently share key functional elements with these tissues. Conversely, failure of immune privilege is a significant cause of disease in the male tract, leading to chronic inflammation, infertility, and pain [20].

CONCLUSIONS

The testis exhibits a distinctive form of immune privilege to protect the germ cells from the host immune attack. The testis presents a special immunological environment, considering its property of immune privilege that tolerates allo- and auto-antigens. Male germ cells are immunogenic. To protect the male germ cells from detrimental immune responses, the testis has adapted an immunoprivileged environment. Therefore, multiple immunosuppressive factors are involved in the maintenance of immune homeostasis in testicular tissue. The testicular properties that provide immune privilege can also protect auto-antigenic germ cells from detrimental immune responses. Androgens also regulate the testicular immunoprivileged status. However, the testis locally generates an efficient innate immune system against pathogens. The testis is immunologically privileged but also immunologically fragile organ.

Author Contributions

M.C. conceived the original draft preparation. M.C. and I.M.C. were responsible for conception and design of the review. M.C. and I.M.C. were responsible for the data acquisition and for the collection and assembly of the articles/published data, and their inclusion and interpretation in this review. All authors contributed to the critical revision of the manuscript for valuable intellectual content. All authors have read and agreed with the final version of the manuscript.

Compliance with Ethics Requirements

The authors declare no conflict of interest regarding this article.

The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law.

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