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Viewpoint

Blocking natural killer cells in testicular torsion may prevent autoimmunity against low expressing major histocompatibility complex class I germ cells

Immune-privileged sites in the body are places where foreign antigens do not elicit an inflammatory immune response, but are tolerated, with the aim of protecting the organ from the detrimental consequences of immune response[1,2]. The presence of immune privilege in the testis protects autoimmune destruction of auto-immunogenic germ cells[1]. Impairment of immune privilege in the testis, which occurs during infection, inflammation and trauma, has been associated with an immune response and autoimmunity against germ cells in both testes and infertility[3].

Testicular torsion is a surgical emergency, and the golden time for the salvage of the twisted testis is eight hours at most[4]. Ipsilateral torsion is frequently associated with the destruction of germinal cells in contralateral testis and infertility[4]. The underlying mechanisms for the contralateral testis injury and infertility need to be identified. Ischaemic-reperfusion injury is a suggestive mechanism, but blocking this pathway does not have any therapeutic application[5]. Immunological responses against germ cells contributed to infertility in the torsion as well as in unilateral vasectomy and a variety of male infertility[4,6,7]. Immunization of male animal models with sperm proteins has been found to be associated with anti-sperm antibody and infertility[7]. Due to the inadequate knowledge on underlying mechanisms, standard therapy for the testicular torsion remains surgery.

Immunology of the testis

The testis comprises the seminiferous tubules surrounded by an interstitial tissue. The presence of immune privilege in the entire testis partly contributed to the blood-testis barrier (BTB)[8]. The BTB, which is not penetrated by blood and lymphatic vessels, is formed by tight junctions between Sertoli cells in the seminiferous epithelium. The BTB provides such a strict physical barrier that even certain small dyes are not able to enter the seminiferous tubules[8]. No immune cells have also been detected in the seminiferous tubules under physiological conditions. The BTB physically isolates the main events of spermatogenesis[8]. The barrier divides the seminiferous tubule into the basal and adluminal compartments. The early stages of spermatogenesis take place in the basal compartment with some contacts with the testis interstitium. The late stages of spermatogenesis take place in the adluminal compartment, which is behind the barrier. The existence of auto-antigenic germ cells outside the BTB (in the basal compartment) without generating an immune response indicates the presence of an immune privilege outside the barrier within the testis interstitium. In fact, the whole testis is immune-privileged rather than just the BTB[8].

In addition to the contribution of physical barriers to the immune suppression in the testis, other factors are also indispensable to sustain the tolerance such as the secretion of immunoregulatory factors by non-immune cells, notably Sertoli cells, unique pattern of immune cells within the testis interstitium and diminished expression of major histocompatibility complex class-I (MHC-I) molecules on male germ cells[1,3,8].

Sertoli cells actively suppress the immune response in the seminiferous tubule and also in the testis interstitium. Sertoli cells produce transforming growth factor beta (TGF-β), indoleamine 2-3-dioxygenase, activin A, galectin-1, several complement inhibitors and serine protease inhibitors, an array of immune-modulatory molecules that suppress the immune response[8].

Immune cells in testis are different from those in the peripheral blood. In primates, these include macrophages (~49%), T-lymphocytes (30.8%) with

CD4 and CD8 T-lymphocyte proportions similar to those in the blood, granulocytes (3.3%) and B-lymphocytes (0.24%). Small populations of myeloid and plasmacytoid dendritic cells, natural killer (NK) cells and NKT cells were also reported[9]. The functions of macrophages and T-lymphocytes in the testis have been well studied, and their significant trends towards immune suppression have been verified[9,10].

Male germ cells do not express MHC-I, the major receptor for binding to CD8 on the surface of cytotoxic T- lymphocytes[3]. While the absence of MHC-I molecules on germ cells protects them from CD8+ T Lymphocytes, it makes germ cells vulnerable to destruction by NK cells, a group of innate immune cells that are the main killers of tumour cells and virus-infected cells with diminished expression of MHC -I molecules[11].

Natural killer (NK) cells in general and in the testis

NK cells are divided into two main subpopulations based on CD56 and CD16 surface markers. CD16, the Fc receptor for IgG, is involved in antibody-dependent cell-mediated cytotoxicity[11]. The mature subtype of $CD56^{dim}$ $CD16^{bright}$ constitutes 90 per cent of the NK cell population in the peripheral blood and is highly cytotoxic with a large amount of granzyme B and perforin. However, the more immature subset of $CD56^{bright}$ $CD16^{dim}$ has minimal cytotoxic capacity and constitutes the prominent population in secondary lymphoid organs[11].

NK cells express a variety of activating and inhibitory receptors whose expressions tightly regulate NK cell functions. The main inhibitory receptors are killer immunoglobulin-like receptors (KIR or CD158), NKG2A and leukocyte inhibitory receptors (LIR1, LAIR-1). Their main ligands are MHC-I molecules[11], meaning that the absence of MHC-I molecules removes inhibition from NK cells; however, even after removal of inhibition, NK cells need an activating signal. The main activating receptor for NK cells is NKG2D which can bind to major histocompatibility complex class I chain-related protein A/B (MICA/B) and UL16 binding proteins (ULPBs)[11]. The mRNA of ULBPs and MICA/B[12], but not protein[13], has been detected in normal testis. Their expressions have not been investigated under pathological conditions in the testis. These molecules particularly MICA/B are not expressed in normal tissues and most cells are able to express them at protein level only under various stress conditions[2].

The phenotypes of NK cells have been extensively studied in some immune-privileged sites, such as the brain and placenta, and are shown to be quantitatively and qualitatively different from those in the peripheral blood. The dominant subpopulation is $CD56^{brigh}$ NK cells with immunosuppressive functions, and changing in subpopulations has been associated with breaking in the organ tolerance and certain pathology[2]. For example, in the placenta, NK cells are predominantly $CD56^{brigh}$ cells and express significant higher levels of inhibitory receptors, such as NKG2A, leukocyte immunoglobulin-like receptor subfamily B member 1 and killer-cell immunoglobulin-like receptors, compared to those in the peripheral blood[2]. In the testis, the subpopulations of NK cells and their functions have not been thoroughly studied yet[1] that might be due to the low percentage of these cells in physiological conditions in the testis.

NK cell activation not only directly destroys cells with diminished expression of MHC-I but also it is suggested that NK-cell-mediated lysis of target cells is a source of apoptotic bodies for uptake by antigen presenting cells, which may promote antigen presentation to CD4+ T-cells boosting other arms of the immune response[14]. NK cells are required for some aspects of antibody production as has been shown in NK cell-deficient mice lacking certain antibody production[15]. Moreover, NK cells are main immune cells contributed to immune surveillance against cancer[2,14].

Hypothesis

To date, no drug therapy as an addition to surgery has been successfully used in the testicular torsion[5]. The possible manipulation of NK cells in the torsion has not been investigated yet. There are some clues towards the involvement of NK cells in the germ cell loss in the torsion:

(*i*) Shifting in subpopulations of NK-cells and CD4+ T-cells in the testis, but not CD8+ cytotoxic cells, in testicular torsion, and its correlation with antisperm antibodies and infertility[4]. The author did not provide further information on what NK cell markers they have investigated.

(*ii*) In the testicular torsion, proteomic study of rat testis indicated the upregulation of killer cell lectin-like receptor[16] which can be an NK cell activating receptor[17].

(*iii*) Increase of CD56+ NK cells in the semen of a subgroup of subfertile men[18].

We hypothesize that due to the increased vascular permeability during testicular torsion[5] cytotoxic NK cells in the peripheral blood temporary leak into the testis, and the exposure of low-expressing MHC class-I germ cells to these cytotoxic NK cause immediate direct destruction of these cells. Destruction of germ cells releases antigens which might be presented to CD4+ lymphocytes and lead to autoantibody production and further destruction of germ cells in the other testis. It is worth mentioning that NK cells are required for an efficient IgG2a production in mice models[15]. The analysis of testicular torsion side effects in NK cell-deficient mice will determine the effect of NK cells on the outcome of the disease and would verify our hypothesis.

Application of hypothesis

Blocking NK cells would prevent disease progression in NK-mediated pathological conditions. For example, during the developmental stages of type 1 diabetes, blocking NK cell activating receptors has been shown to inhibit the disease progression in animal models[14]. If our hypothesis about the role of NK cells in germ cell destruction in the testicular torsion is true, then blocking NK cells in testicular torsion may prevent or reduce the immune response against the germ cells and risk of infertility. This intervention should be an addition to surgical procedure and immediate after the diagnosis, but for a short period until the inflammation is resolved, at least from the theoretical point of view. Possible side effect of our suggestive therapy might be some temporary defects in antibody response[15] or cancer immune survilance[2,14].

Conclusion

The lack of expression of MHC-I molecules makes germ cells vulnerable to the destruction by NK cells. We hypothesize that cytotoxic NK cells in the peripheral blood leak into testis during testicular torsion and destroy germ cells, and thus blocking NK cells may reduce the immune response, autoimmunity, destruction of germ cells and subsequent infertility.

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References

1. Zhao S, Zhu W, Xue S, Han D. Testicular defense systems: Immune privilege and innate immunity. *Cell Mol Immunol* 2014; *11* : 428-37.
2. Bolourian A, Mojtahedi Z. Possible damage to immune-privileged sites in natural killer cell therapy in cancer patients: Side effects of natural killer cell therapy. *Immunotherapy* 2017; *9* : 281-8.
3. Fijak M, Meinhardt A. The testis in immune privilege. *Immunol Rev* 2006; *213* : 66-81.
4. Rybkiewicz M. Long-term and late results of treatment in patients with a history of testicular torsion. *Ann Acad Med Stetin* 2001; *47* : 61-75.
5. Feher AM, Bajory Z. A review of main controversial aspects of acute testicular torsion. *J Acute Dis* 2016; *5* : 1-8.
6. Rival C, Wheeler K, Jeffrey S, Qiao H, Luu B, Tewalt EF, *et al.* Regulatory T cells and vasectomy. *J Reprod Immunol* 2013; *100* : 66-75.
7. Bandivdekar AH. Development of antifertility vaccine using sperm specific proteins. *Indian J Med Res* 2014; *140* (Suppl): S73-7.
8. Kaur G, Thompson LA, Dufour JM. Sertoli cells - Immunological sentinels of spermatogenesis. *Semin Cell Dev Biol* 2014; *30* : 36-44.
9. De Rose R, Fernandez CS, Hedger MP, Kent SJ, Winnall WR. Characterisation of macaque testicular leucocyte populations and T-lymphocyte immunity. *J Reprod Immunol* 2013; *100* : 146-56.
10. Winnall WR, Hedger MP. Phenotypic and functional heterogeneity of the testicular macrophage population: A new regulatory model. *J Reprod Immunol* 2013; *97* : 147-58.
11. Del Zotto G, Marcenaro E, Vacca P, Sivori S, Pende D, Della Chiesa M, *et al.* Markers and function of human NK cells in normal and pathological conditions. *Cytometry B Clin Cytom* 2017; *92* : 100-14.
12. Cerwenka A. New twist on the regulation of NKG2D ligand expression. *J Exp Med* 2009; *206* : 265-8.
13. Ghadially H, Brown L, Lloyd C, Lewis L, Lewis A, Dillon J, *et al.* MHC class I chain-related protein A and B (MICA and MICB) are predominantly expressed intracellularly in tumour and normal tissue. *Br J Cancer* 2017; *116* : 1208-17.
14. Mandal A, Viswanathan C. Natural killer cells: In health and disease. *Hematol Oncol Stem Cell Ther* 2015; *8* : 47-55.
15. Satoskar AR, Stamm LM, Zhang X, Okano M, David JR, Terhorst C, *et al.* NK cell-deficient mice develop a Th1-like response but fail to mount an efficient antigen-specific IgG2a antibody response. *J Immunol* 1999; *163* : 5298-302.

16. Ouh IO, Seo MG, Shah FA, Gim SA, Koh PO. Proteomic analysis of testicular ischemia-reperfusion injury in rats. *J Vet Med Sci* 2014; *76* : 313-21.
17. Montaldo E, Vitale C, Cottalasso F, Conte R, Glatzer T, Ambrosini P, *et al.* Human NK cells at early stages of differentiation produce CXCL8 and express CD161 molecule that functions as an activating receptor. *Blood* 2012; *119* : 3987-96.
18. Seshadri S, Flanagan B, Vince G, Lewis-Jones DJ. Detection of subpopulations of leucocytes in different subgroups of semen sample qualities. *Andrologia* 2012; *44* (Suppl 1): 354-61.