Received: 20 April 2018 | Revised: 25 June 2018 | Accepted: 12 July 2018

DOI: 10.1111/and.13120

INVITED REVIEW

WILEY ANDROLOGIA

Exploring the role of antigen presenting cells in male genital tract

Vanesa A. Guazzone[1,2]

[1]Universidad de Buenos Aires, Facultad de Medicina, Departamento de Biología Celular e Histología/Unidad Académica II., Buenos Aires, Argentina

[2]Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad de Buenos Aires , Instituto de Investigaciones Biomédicas (INBIOMED), Buenos Aires, Argentina

Correspondence
Vanesa A. Guazzone, INBIOMED UBA-CONICET, Facultad de Medicina, Paraguay 2155 piso 10, Ciudad Autónoma de Buenos Aires, Argentina (C1121ABG).
Email: vaguazzone@yahoo.com.ar

Abstract

Antigen presenting cells (APCs) are a critical mediator between innate and adaptive immune response. APCs have diverse functions in physiological and pathological conditions, such as maintenance of tissue homoeostasis, prevention of autoimmunity and defence against pathogenic microorganisms and cancer cells. Dendritic cells (DCs) and macrophages (Mϕs) are "professional" APCs that internalise and process allo- and autoantigens; then, resulting peptides are exhibited together with major histocompatibility complex (MHC) molecules expressed at the cell surface. MHC-antigen complexes are presented to "naïve" T cells, thereby stimulating proliferation and differentiation of effector and regulatory T cells. The aim of this review was to summarise current understanding of DCs and Mϕs in testis and epididymis. Male reproductive tract environment is characterised by contradictory needs for tolerance against autoantigenic germ cells that appear after the establishment of central tolerance, and the capacity to mount pro-inflammatory innate immune responses against a wide array of sexually transmitted pathogens. Therefore, exploration of the role of APCs in male reproductive organs is helpful to understand mechanisms of male infertility associated with disruption of the delicate equilibrium between immune privilege and inflammation.

KEYWORDS

dendritic cells, epididymis, macrophages, testis

1 | INTRODUCTION

The immune system possesses an innate immune system which recognises pathogens through evolution preserved, pathogen associated molecular patterns and an adaptive immune system which specifically recognises MHC molecules, loaded with allo- or autoantigens, and having developed immunological memory. The cell types of the adaptive immune system are B and T cells and the "professional" antigen-presenting cells (APCs). To be classified as a "professional" APC, a cell should be able to acquire and process antigens and also exhibit accessory molecules which enable them to interact with T cells, thereby providing efficacy, specificity and memory to immune response. $CD8^+$ and $CD4^+$ T cell express clonally distributed receptors that recognise antigens associated with MHC class I and II molecules respectively. Activated APCs can also secrete various cytokines that drive polarisation of T cells into different effector cells or regulatory cells. This review focuses on understanding of dendritic cells (DCs) and macrophages (Mϕs) in male genital tract, with specific emphasis on their role as APCs in testis and epididymis. We also compare the phenotype and function of APCs in physiological and pathological states.

2 | APC IN TESTIS

Spermatozoa represent an immunologic challenge for the immune system because spermatic antigens appear at puberty long after the establishment of central tolerance. Central tolerance refers to the

process by which potentially self-reactive lymphocytes are deleted in the thymus and in bone marrow during foetal development and early life (Mold & McCune, 2012). In order to avoid autoimmune responses that would be detrimental to male fertility, the adaptive immune system must be efficiently silenced.

The normal mammalian testis contains diverse immune cells within the interstitial compartment comprising T cells, mast cells, natural killer cells, DCs and Mϕs (Pérez et al., 2013). The number and distribution of immune cells differ between mammalian species, potentially reflecting differences in lifespan, functional activity and exposure to pathogens.

In mice testis, two different Mϕ populations have been identified: the interstitial yolk sac-derived Mϕs (major histocompatibility complex class II (MHCII)-negative) and the peritubular (MHCII-positive) postnatal bone marrow-derived Mϕs (DeFalco et al., 2015; Mossadegh-Keller et al., 2017). Once established, these two populations exhibit long lifespans and low turnover in the adult testis in the steady state. Phenotypic characterisation of interstitial and peritubular Mϕ showed highly similar gene expression profiles with the absence of proinflammatory gene expression but with expression of a large number of immunosuppressive and alternative M2-type activation genes. As an exception to this, the expression of only a few genes differed, such as interleukin (IL)10 and Mϕ receptor with collagenous structure (MARCO), which were more highly expressed in interstitial Mϕ, and IL1β and factors regulating antigen processing and presentation, which was increased in peritubular Mϕ (Mossadegh-Keller et al., 2017). This suggests that peritubular Mϕ might have antigen-presenting activity playing a unique tolerising role against some meiotic germ cell antigens that egress from seminiferous tubules (Tung et al., 2017).

M2 Mϕs are characterised by secretion of high amounts of anti-inflammatory cytokines (IL10, transforming growth factor-beta (TGFβ) and low levels of pro-inflammatory cytokines (IL12, tumour necrosis factor-alpha (TNFα)) and by the expression of the scavenger receptor CD163. In contrast, M1 Mϕs are defined by very high secretion of pro-inflammatory cytokines (IL6, TNFα and IL12) and production of reactive oxygen species (ROS) and nitric oxide (NO), which facilitates clearance of microbial pathogens (Bhushan & Meinhardt, 2017).

Testicular microenvironment could control Mϕ identity. Wang et al. (2017) showed that rat testicular interstitial fluid (IF) shifted blood monocytes stimulated by Granulocyte Macrophage Colony-Stimulating Factor (GMCSF) (M1 phenotype) towards the M2 phenotype. IF-polarised M2 Mϕs mimic properties of testicular Mϕs, such as increased expression of CD163, high secretion of IL10 and low secretion of TNFα. IF-polarised Mϕs also display immunoregulatory functions by inducing expansion of immunosuppressive regulatory T cells (Tregs).

DCs, distinguishable from Mϕs by their expression of specific markers, were identified for the first time in the rat testicular interstitial space by monoclonal antibodies OX-62 and CD11c (Rival, Lustig, et al., 2006). The anti-OX-62 antibody recognises the E2 integrin alpha chain, whereas the anti-CD11c antibody is specific for the integrin alpha x chain. Isolated testicular DCs and DCs from testicular draining lymph nodes (LN) do not activate T cells in physiological conditions, suggesting that the testicular microenvironment may promote DC differentiation into tolerogenic DCs (Guazzone et al., 2011; Rival et al., 2007). DCs promote peripheral tolerance by several mechanisms such as generation of Tregs and induction of T-cell anergy/deletion (Manicassamy & Pulendran, 2011). Gao et al. (2016) reported that Sertoli cells mediate the immunosuppressive activity of bone marrow-derived DCs because in vitro exposure to Sertoli cells downregulated expression levels of costimulatory molecules on DCs and also reduced production of IL12p70 and TNFα in DCs after lipopolysaccharide stimulation. Moreover, Sertoli cell-conditioned DCs inhibited T-cell proliferation but promoted Treg development.

In view of the previous description, APCs in healthy testis exhibit relatively low inflammatory responses and high immunosuppressive properties. These phenotypes support testicular immune privilege (Zhao, Zhu, Xue, & Han, 2014). However, in pathological conditions or injury, different Mϕ and DC populations may possess unique immunological abilities. The pathophysiological role of testicular Mϕs and DCs is further underlined by the elevated numbers of Mϕ and DCs demonstrated in human seminomas (reviewed by Loveland et al., 2017), in biopsy samples of infertile patients with Sertoli cell only syndrome (Frungieri et al., 2002), and in azoospermic testis with chronic inflammation (Duan et al., 2011) respectively. The contribution of APCs to testicular inflammation was studied in more detail in animal models of chronic orchitis.

Interstitial Mϕs and DCs are critical participants in experimental autoimmune orchitis (EAO); in vivo depletion of these cells in rat EAO by clodronate-containing liposomes significantly reduces EAO incidence and severity (Rival et al., 2008). In orchitis, Mϕs M1-like significantly infiltrate the testis and are detrimental to spermatogenesis. They express high levels of MHC class II and costimulatory molecules CD80 and CD86; they also produce proinflammatory cytokines, mainly TNFα, IL6, interferon-gamma (IFNγ) and NO (Jarazo Dietrich et al., 2015; Jarazo-Dietrich et al., 2012; Rival, Theas, Guazzone, & Lustig, 2006; Suescun, Rival, Theas, Calandra, & Lustig, 2003). The expression pattern of MHCII and costimulatory molecules suggests their antigen-presenting activity.

The DC maturation state is a control point for induction of peripheral tolerance or autoimmunity. The maturation process encompasses downregulation of endocytic capacity and upregulation of MHC and costimulatory molecules (CD80, CD86, and CD40) along with chemokine receptors. In this context, mature DCs migrate to LN, release the corresponding polarising cytokines and stimulate specific T cells.

Interestingly, similar levels of CD80, CD86 and MHC class II are observed in DCs isolated from normal and chronically inflamed rat testis. However, DCs isolated from EAO testis upregulate mRNA expression of chemokine receptor CCR7 and expression of IL10 and IL12p35. Upregulation of the chemokine receptor CCR7 is a key step required for the entry of DC into LN and their homing to T- and B-cell zones therein. Functionally, testicular EAO-DC and DC from draining LN of EAO testis, but not those from nondraining

LN, are able to stimulate T-cell proliferation (Guazzone et al., 2011; Rival et al., 2007).

3 | APC IN EPIDIDYMIS

In contrast to the immunologically privileged testis, the epididymis does not support prolonged allogeneic graft survival (Kazeem, 1988). Furthermore, its epithelial tight junctions may not be as effective as those of the blood–testis barrier, and direct interactions between intra-epithelial immune cells and either sperm antigens or ascending pathogens is possible (Michel, Pilatz, Hedger, & Meinhardt, 2015). Lymphomononuclear cells are found in all regions of the steady state epididymis, and their distribution is highly organised. Unlike the testis where APCs are excluded from the seminiferous epithelium, in other regions of the male genital tract such as the epididymis, the vas deferens, the prostate and the seminal vesicles, they are present within the epithelial wall (Hedger, 2011).

Using transgenic mouse models expressing fluorescent proteins under the control of the CD11c (integrin alpha X chain) and CX3CR1 (fractalkine receptor), two markers of mononuclear phagocytes, Da Silva et al. (2011) described a very abundant and heterogeneous population of Mϕs and DCs in the peritubular and interstitial compartments of mouse epididymis. Moreover, Shum et al. (2014) confirmed that epididymal CD11c+ DCs and F4/80+ expressing Mϕs located in the basal region of the epithelium establish close interactions with neighbouring epithelial cells, particularly in the initial segment, where they project intraepithelial dendrites. And recently, Voisin et al. (2018), using a wide set of markers and flow cytometry, demonstrated that APCs are more abundant in *caput* than in *cauda* epididymis. Unlike the dense network of CD11c+ cells localised in normal mouse epididymis, human studies suggest that the number of epididymal CD11c+ cells is relatively low and that they are found in the interstitial compartment of epididymis but not in the epithelium. Only in chronic epididymal inflammation did the number of CD11c+ cells increase in the infiltrate and in epididymal epithelium (Duan et al., 2016).

Functionally, DCs isolated from normal mouse epididymis have antigen-presenting activity in vitro (Da Silva et al., 2011). The in vivo activity of the epididymal DCs was further illustrated by this group's report that after efferent duct ligation (known to provoke apoptosis in the epididymal proximal epithelium), epididymal DCs phagocytose and eliminate apoptotic cells to maintain the integrity of the epididymis tubule (Smith et al., 2014). While doing so, they observed that DC dendrites retract. These behaviours at least illustrate the phagocytic ability of DCs in the very proximal segment of the epididymis (Guiton, Henry-Berger, & Drevet, 2013).

4 | FUTURE PERSPECTIVES

In mammals, immunological tolerance results from fundamentally distinct mechanisms that involve both innate and adaptive immunity. "Professional" APCs such as DCs and Mϕs are able to phagocytose, process, and load MHC molecules with exogenous proteins from pathogenic microorganisms and also endogenous proteins including germ cells. MHC-antigen complexes are presented to "naïve" T cells which stimulate proliferation and differentiation of different T-cells subsets depending on the costimulatory molecules engaged and cytokines produced. Despite their abundance and heterogeneity, APC represent only a few pieces in the immunological puzzle that is the testis and epididymis. As the functions of testicular and epididymal non-immune cells in immune control are not yet totally understood, the role of Sertoli cells and epididymal epithelial cells as APCs should be elucidated in detail.

5 | CLINICAL IMPLICATIONS

DCs and Mϕs are APCs having diverse immunoregulatory functions in physiological and pathological conditions, such as maintenance of tissue homeostasis, induction of peripheral tolerance and defence against pathogenic microorganisms and cancer antigens. Therefore, unravelling immune mechanisms in male reproductive tract that prevent the development of immune response to germ cells while maintaining the ability to start competent immune response against pathogens and cancer cells will provide exceptional knowledge on therapeutic approaches for human epididymis and chronic testis inflammation and autoimmunity associated with subfertility and infertility.

ORCID

Vanesa A. Guazzone http://orcid.org/0000-0001-9070-2388

REFERENCES

Bhushan, S., & Meinhardt, A. (2017). The macrophages in testis function. *Journal of Reproductive Immunology*, *119*, 107–112. https://doi.org/10.1016/j.jri.2016.06.008

Da Silva, N., Cortez-Retamozo, V., Reinecker, H. C., Wildgruber, M., Hill, E., Brown, D., … Breton, S. (2011). A dense network of dendritic cells populates the murine epididymis. *Reproduction*, *141*(5), 653–663. https://doi.org/10.1530/REP-10-0493

DeFalco, T., Potter, S. J., Williams, A. V., Waller, B., Kan, M. J., & Capel, B. (2015). Macrophages contribute to the spermatogonial niche in the adult testis. *Cell Reports*, *12*(7), 1107–1119. https://doi.org/10.1016/j.celrep.2015.07.015

Duan, Y. G., Wang, P., Zheng, W., Zhang, Q., Huang, W., Jin, F., & Cai, Z. (2016). Characterisation of dendritic cell subsets in chronically inflamed human epididymis. *Andrologia*, *48*, 431–440. https://doi.org/10.1111/and.12463

Duan, Y. G., Yu, C. F., Novak, N., Bieber, T., Zhu, C. H., Schuppe, H. C., … Allam, J. P. (2011). Immunodeviation towards a Th17 immune response associated with testicular damage in azoospermic men. *International Journal of Andrology*, *34*, e536–e545. https://doi.org/10.1111/j.1365-2605.2010.01137.x

Frungieri, M. B., Calandra, R. S., Lustig, L., Meineke, V., Kohn, F. M., Vogt, H. J., & Mayerhofer, A. (2002). Number, distribution pattern, and identification of macrophages in the testes of infertile

men. *Fertility and Sterility*, *78*, 298–306. https://doi.org/10.1016/S0015-0282(02)03206-5

Gao, J., Wang, X., Wang, Y., Han, F., Cai, W., Zhao, B., … Hu, D. (2016). Murine Sertoli cells promote the development of tolerogenic dendritic cells: A pivotal role of galectin-1. *Immunology*, *148*, 253–265. https://doi.org/10.1111/imm.12598

Guazzone, V. A., Hollwegs, S., Mardirosian, M., Jacobo, P., Hackstein, H., Wygrecka, M., … Fijak, M. (2011). Characterization of dendritic cells in testicular draining lymph nodes in a rat model of experimental autoimmune orchitis. *International Journal of Andrology*, *34*, 276–289. https://doi.org/10.1111/j.1365-2605.2010.01082.x

Guiton, R., Henry-Berger, J., & Drevet, J. R. (2013). The immunobiology of the mammalian epididymis: The black box is now open!. *Basic and Clinical Andrology*, *23*, 8. https://doi.org/10.1186/2051-4190-23-8

Hedger, M. P. (2011). Immunophysiology and pathology of inflammation in the testis and epididymis. *Journal of Andrology*, *32*(6), 625–640. https://doi.org/10.2164/jandrol.111.012989

Jarazo Dietrich, S., Fass, M. I., Jacobo, P. V., Sobarzo, C. M., Lustig, L., & Theas, M. S. (2015). Inhibition of NOS-NO system prevents autoimmune orchitis development in rats: relevance of NO released by testicular macrophages in germ cell apoptosis and testosterone secretion, *PLoS One*, *10*(6), e0128709. https://doi.org/10.1371/journal.pone.0128709

Jarazo-Dietrich, S., Jacobo, P., Pérez, C. V., Guazzone, V. A., Lustig, L., & Theas, M. S. (2012). Up regulation of nitric oxide synthase-nitric oxide system in the testis of rats undergoing autoimmune orchitis. *Immunobiology*, *217*, 778–787. https://doi.org/10.1016/j.imbio.2012.04.007

Kazeem, A. A. (1988). A critical consideration of the rat epididymis as an immunologically privileged site. *Scandinavian Journal of Immunology*, *27*, 149–156. https://doi.org/10.1111/j.1365-3083.1988.tb02333.x

Loveland, K. L., Klein, B., Pueschl, D., Indumathy, S., Bergmann, M., Loveland, B. E., … Schuppe, H. C. (2017). Cytokines in male fertility and reproductive pathologies: immunoregulation and beyond. *Frontiers in Endocrinology*, *8*, 307. https://doi.org/10.3389/fendo.2017.00307

Manicassamy, S., & Pulendran, B. (2011). Dendritic cell control of tolerogenic responses. *Immunological Reviews*, *241*(1), 206–227. https://doi.org/10.1111/j.1600-065X.2011.01015.x

Michel, V., Pilatz, A., Hedger, M. P., & Meinhardt, A. (2015). Epididymitis: Revelations at the convergence of clinical and basic sciences. *Asian Journal of Andrology*, *17*(5), 756–763. https://doi.org/10.4103/1008-682X.155770

Mold, J. E., & McCune, J. M. (2012). Immunological tolerance during fetal development: From mouse to man. *Advances in Immunology*, *115*, 73–111. https://doi.org/10.1016/B978-0-12-394299-9.00003-5

Mossadegh-Keller, N., Gentek, R., Gimenez, G., Bigot, S., Mailfert, S., & Sieweke, M. H. (2017). Developmental origin and maintenance of distinct testicular macrophage populations. *Journal of Experimental Medicine*, *214*(10), 2829–2841. https://doi.org/10.1084/jem.20170829

Pérez, C. V., Theas, M. S., Jacobo, P. V., Jarazo-Dietrich, S., Guazzone, V. A., & Lustig, L. (2013). Dual role of immune cells in the testis: Protective or pathogenic for germ cells? *Spermatogenesis*, *3*(1), e23870. https://doi.org/10.4161/spmg.23870

Rival, C., Guazzone, V. A., von Wulffen, W., Hackstein, H., Schneider, E., Lustig, L., … Fijak, M. (2007). Expression of co-stimulatory molecules, chemokine receptors and proinflammatory cytokines in dendritic cells from normal and chronically inflamed rat testis. *Molecular Human Reproduction*, *13*, 853–861. https://doi.org/10.1093/molehr/gam067

Rival, C., Lustig, L., Iosub, R., Guazzone, V. A., Schneider, E., Meinhardt, A., & Fijak, M. (2006). Identification of a dendritic cell population in normal testis and in chronically inflamed testis of rats with autoimmune orchitis. *Cell and Tissue Research*, *324*(2), 311–318.

Rival, C., Theas, M. S., Guazzone, V. A., & Lustig, L. (2006). Interleukin-6 and IL-6 receptor cell expression in testis of rats with autoimmune orchitis. *Journal of Reproductive Immunology*, *70*, 43–54.

Rival, C., Theas, M. S., Suescun, M. O., Jacobo, P., Guazzone, V. A., van Rooijen, N., & Lustig, L. (2008). Functional and phenotypic characteristics of testicular macrophages in experimental autoimmune orchitis. *The Journal of Pathology*, *215*, 108–117. https://doi.org/10.1002/path.2328

Shum, W. W., Smith, T. B., Cortez-Retamozo, V., Grigoryeva, L. S., Roy, J. W., Hill, E., … Da Silva, N. (2014). Epithelial basal cells are distinct from dendritic cells and macrophages in the mouse epididymis. *Biology of Reproduction*, *90*(5), 1–10. https://doi.org/10.1095/biolreprod.113.116681

Smith, T. B., Cortez-Retamozo, V., Grigoryeva, L. S., Hill, E., Pittet, M. J., & Da Silva, N. (2014). Mononuclear phagocytes rapidly clear apoptotic epithelial cells in the proximal epididymis. *Andrology*, *2*(5), 755–762. https://doi.org/10.1111/j.2047-2927.2014.00251.x

Suescun, M. O., Rival, C., Theas, M. S., Calandra, R. S., & Lustig, L. (2003). Involvement of tumor necrosis factor-alpha in the pathogenesis of autoimmune orchitis in rats. *Biology of Reproduction*, *68*, 2114–2121. https://doi.org/10.1095/biolreprod.113.116681

Tung, K. S. K., Harakal, J., Qiao, H., Rival, C., Li, J. C. H., Paul, A. G. A., … Goldberg, E. (2017). Egress of sperm autoantigen from seminiferous tubules maintains systemic tolerance. *Journal of Clinical Investigation*, *127*, 1046–1060. https://doi.org/10.1172/JCI89927

Voisin, A., Whitfield, M., Damon-Soubeyrand, C., Goubely, C., Henry-Berger, J., Saez, F., … Guiton, R. (2018). Comprehensive overview of murine epididymal mononuclear phagocytes and lymphocytes: Unexpected populations arise. *Journal of Reproductive Immunology*, *126*, 11–17. https://doi.org/10.1016/j.jri.2018.01.003

Wang, M., Fijak, M., Hossain, H., Markmann, M., Nüsing, R. M., Lochnit, G., … Bhushan, S. (2017). Characterization of the Micro-Environment of the Testis that Shapes the Phenotype and Function of Testicular Macrophages. *Journal Immunology*, *198*(11), 4327-4340. https://doi.org/10.4049/jimmunol.1700162

Zhao, S., Zhu, W., Xue, S., & Han, D. (2014). Testicular defense systems: Immune privilege and innate immunity. *Cellular & Molecular Immunology*, *11*(5), 428–437. https://doi.org/10.1038/cmi.2014.38

How to cite this article: Guazzone VA. Exploring the role of antigen presenting cells in male genital tract. *Andrologia*. 2018;50:e13120. https://doi.org/10.1111/and.13120