Asian Journal of Andrology (2015) 17, 591–596

www.asiaandro.com; www.ajandrology.com

Open Access

INVITED REVIEW

Sperm Biology

Exploring the role of mononuclear phagocytes in the epididymis

Nicolas Da Silva, Tegan B Smith

The onslaught of foreign antigens carried by spermatozoa into the epididymis, an organ that has not demonstrated immune privilege, a decade or more after the establishment of central immune tolerance presents a unique biological challenge. Historically, the physical confinement of spermatozoa to the epididymal tubule enforced by a tightly interwoven wall of epithelial cells was considered sufficient enough to prevent cross talk between gametes and the immune system and, ultimately, autoimmune destruction. The discovery of an intricate arrangement of mononuclear phagocytes (MPs) comprising dendritic cells and macrophages in the murine epididymis suggests that we may have underestimated the existence of a sophisticated mucosal immune system in the posttesticular environment. This review consolidates our current knowledge of the physiology of MPs in the steady state epididymis and speculates on possible interactions between auto-antigenic spermatozoa, pathogens and the immune system by drawing on what is known about the immune system in the intestinal mucosa. Ultimately, further investigation will provide valuable information regarding the origins of pathologies arising as a result of autoimmune or inflammatory responses in the epididymis, including epididymitis and infertility.

Asian Journal of Andrology (2015) **17**, 591–596; doi: 10.4103/1008-682X.153540; published online: 11 May 2015

Keywords: antigen-presenting cells; autoimmunity; dendritic cells; epididymis; macrophages; peripheral tolerance; sperm maturation; spermatozoa

MONONUCLEAR PHAGOCYTES

The murine epididymis, which is the primary site of sperm maturation and storage, contains an intricate network of mononuclear phagocytes (MPs) comprising dendritic cells (DCs) and macrophages (Mϕs), which are key regulators of innate and adaptive immunity in numerous organ systems.[1–5] The ontogeny and functions of MPs have been studied extensively in lymphoid and nonlymphoid organs, particularly in the small intestine mucosa.[6–9] In contrast, the epididymis remains poorly studied and, therefore, the role of MPs in this largely uncharacterized mucosal system remains mostly speculative. This review will describe what is currently known about MPs in the epididymal mucosa and explore the possible roles of epididymal MPs (eMPs) based on comparisons with the small intestine.

Dendritic cells and Mϕs are phagocytes and professional antigen-presenting cells (APCs). These two fundamental functions give them the ability to orchestrate innate and adaptive immune responses, by sampling self and nonself antigens, presenting processed peptides to naïve T cells, and secreting immunomodulatory factors. The first characteristic of the MP system is the tremendous heterogeneity of Mϕs and DCs.[6,10] The phenotyping of MP subsets is essentially based on the detection of multiple surface markers using flow cytometry-based techniques. **Table 1** describes a selection of immune cell surface markers that will frequently be mentioned in this review. As most markers are expressed by multiple subsets of cells rather than being cell type-specific, the clear identification of phenotypically and functionally distinct populations of immune cells has become increasingly complicated. For instance, the coexpression of CD11c (integrin alpha X chain) and major histocompatibility complex (MHC) class II has been largely used to identify DCs in lymphoid tissues. However, since Mϕs from peripheral nonlymphoid tissues also express CD11c and MHC class II, the distinction between DCs and Mϕs has been at the center of a long and passionate debate in the immunology community. Recent reviews are available to better appreciate the complexity of the MP system.[6,10,11]

MONONUCLEAR PHAGOCYTES IN THE SMALL INTESTINE

In order to better understand of the putative roles of MPs in the epididymis, we will briefly recapitulate the current knowledge of the murine small intestine, which is a well-characterized mucosal system.[11–17] DCs and Mϕs perform complementary functions to maintain epithelial homeostasis and regulate the delicate balance between immune defense and tolerance in the steady state intestine. As potent phagocytes, they clear defective surrounding epithelial cells and sample the luminal compartment by extending dynamic intraepithelial dendrites across the apical tight junction barrier. Intestinal Mϕs are sedentary and mostly derived from circulating Ly 6C (lymphocyte antigen 6C)-high monocytes that enter the lamina propria and ultimately differentiate into CX3CR1$^+$ F4/80$^+$ cells, F4/80 being the archetypal murine Mϕ marker.[11] Recent results have suggested that some resident tissue Mϕs arise from the local self-renewal of fetal precursors that were seeded in organs early during development.[18] The precise mechanisms of MP

Massachusetts General Hospital and Harvard Medical School, Division of Nephrology, Center for Systems Biology, Boston, Massachusetts, USA.
Correspondence: Dr N Da Silva (dasilva.nicolas@mgh.harvard.edu)
This article was presented at the 12th International Symposium on Spermatology, August 10-14, 2014, Newcastle, Australia.

Table 1: Most common cell-surface markers used to identify murine MPs

Marker	*Alternate names*	*Expression and comments*
CD11b	ITGAM, Mac-1	Expressed on numerous leukocytes including monocytes/macrophages, and subsets of DCs
CD11c	ITGAX	Often described as a DC marker, CD11c is also found on subsets of monocytes and macrophages, as well as subsets of lymphocytes, NK cells and neutrophils
CD103	ITGAE	Expressed on intestinal IELs and subsets of DCs. $CD103^+$ DCs are involved in the induction of peripheral tolerance
CX3CR1	Fractalkine/ neurotactine (CX3CL1) receptor	Binds the chemokine CX3CL1. CX3CR1 is expressed on subsets of monocytes, DCs, macrophages, NK cells and T-cells. CX3CR1 has adhesive and migratory functions
F4/80	EMR1 (human)	F4/80 is expressed by murine mature macrophages
MHC class II	MHC class II	Expressed on professional APCs, including DCs and macrophages. Used by APCs to present antigen fragments to naïve T-cells

MPs: mononuclear phagocytes; DCs: dendritic cells; NK: natural killer; IELs: intraepithelial lymphocytes; APCs: antigen-presenting cells; MHC: major histocompatibility complex; ITGAM: integrin alpha M; ITGAX: integrin alpha X; ITGAE: integrin alpha E

replenishment in peripheral tissues are still under intense investigation. In contrast, intestinal DCs are replenished by myeloid precursors via a Flt3L-dependent mechanism and ultimately represent at least four subsets of cells expressing various levels of integrins including CD11b, CD11c and CD103, in addition to other leukocyte markers.[11] Unlike *bona fide* Mϕs, DCs do not express F4/80. Located in the lamina propria of the intestinal epithelium, DCs have been shown to acquire luminal antigens directly,[15–17,19,20] via interaction with goblet cells[14] and/or via interaction with intraepithelial $CX3CR1^+$ Mϕs.[13] In contrast to Mϕs, DCs limit the degradation of phagocytozed particles to preserve antigenicity and are, therefore, specialized in antigen presentation.[21–23] Following antigen uptake and processing, they migrate from the lamina propria to lymph nodes (LNs) to prime and polarize naïve T cells via MHC class II. By inducing the development of $Foxp3^+$ regulatory T cells (Treg), $CD103^+$ DCs initiate the tolerogenic response to food antigens and commensal bacteria.[24] Disruption of the equilibrium between tolerance and defense can result in inflammatory conditions of the gastrointestinal tract tract such as inflammatory bowel disease.[11,25]

To summarize, the steady state small intestinal mucosa contains two major families of phenotypically distinct and functionally complementary MPs. On one hand, Mϕs are in charge of maintaining the integrity of the epithelial barrier and scavenging materials. On the other hand, DCs populate the lamina propria and constitutively acquire luminal antigens directly or indirectly via cell-cell interactions with Mϕs or epithelial cells, and finally migrate into mesenteric LNs (MLNs) to prime naïve T cells and induce either a tolerogenic or an immunogenic response.

FROM AN IMMUNOLOGICAL POINT OF VIEW, IS THE EPIDIDYMIS A SMALL INTESTINE ANALOGUE[26]?

Although the small intestine and the epididymis constitute two radically disparate environments, their respective mucosa may face surprisingly similar immunological challenges. Using the intestine as a model, it seems reasonable that the MPs lining the epididymal duct could be in charge of maintaining the integrity of the epithelial barrier and orchestrating peripheral tolerance to auto-antigenic spermatozoa undergoing maturation; simultaneously, they may fight the pathogens that constantly ascend and threaten the reproductive tract and monitor pathogens circulating in the vasculature **(Figure 1)**.

Sperm are postmeiotic cells that display antigens not expressed anywhere else in the body. In addition, they are abundant (1000 to 2000 sperm enter each human epididymis every second) and appear at puberty, long after the establishment of central tolerance.[27,28] Consequently, male reproductive function relies on strong immunoregulatory mechanisms that prevent sperm from being identified as nonself-cells and rejected by the host's immune system, both in the testis and in the posttesticular environment. In order to control interactions between immune cells and sperm antigens, the reproductive tract is lined by barriers named the "blood-testis barrier" (BTB) and the "blood-epididymis barrier" (BEB).[29] The physiology of the BEB and BTB has been extensively studied, and our readers are kindly advised to consult recent reviews and articles that highlight fundamental differences between the testis and the epididymis.[30–32] Unlike the epididymis, the testis is often considered an "immunologically privileged organ" (i.e., foreign tissues implanted in a testis are not rejected) and, despite their anatomical proximity and their functional complementarity, these two organs seem to constitute two fundamentally distinct immunological environments.[27,28,33,34] Simplistically, the term "barrier" suggests that a physical separation consisting of epithelial cells joined by tight junctions is sufficient to partition sperm from the immune system, thus averting the development of autoimmunity against sperm antigens. In light of recent research, it has become evident that the BTB and the BEB are complex and dynamic structures that do not fully prevent interactions between the immune system and germ cells, but provide support for finely tuned cellular and molecular crosstalk.[31,32,35]

In humans and rodents, the pseudostratified epithelium that lines the epididymal duct is composed of several types of cells, including principal cells, clear/narrow cells, and basal cells (BCs). Altogether, these cells are in charge of creating a succession of microenvironments in which sperm mature and are stored.[36–41] Sperm maturation and storage involves a finely tuned sequence of complex interactions between the epithelium, the luminal fluid, and spermatozoa. Alongside epithelial cells, cells of the immune system were identified in the epididymis several decades ago, including lymphocytes (most likely referred to as "halo cells") and Mϕs.[4,42–45] Both Mϕs and lymphocytes have been shown to respond to changes in the epididymis initiated by aging, vasectomy and infections.[3,46–49] However, these discoveries provide not more than a superficial view of the increasingly complex mucosal immune system composed of many phenotypically, morphologically and functionally distinct subsets of lymphoid and myeloid cells, including MPs, which maintain constant interactions. In order to unravel the immunophysiology of the epididymis, it is now vital to unambiguously identify each subset of immune cell and determine how they interact with their environment. Fortunately, immunologists have developed a battery of powerful tools to study immune cells in other organ systems, thus introducing new perspectives to reproductive biologists. Among those tools, two lines of transgenic mice (CD11c-EYFP[50] and CX3CR1-GFP[51]) have allowed us to illuminate an extensive network of DCs and Mϕs in the mouse epididymis[1] **(Figure 2)**. CD11c (integrin alpha X) and CX3CR1 (a G-protein coupled chemokine receptor) are expressed by several subsets of Mϕs and DCs.

Although Mϕs have been described in the murine epididymis in the past, these mouse models revealed the density of the MP network and its complex interactions with the duct, highlighting the fact that the mucosal immune system present in the epididymis is more sophisticated than generally documented in the literature. MPs line the excurrent duct in all epididymal segments (as well as efferent

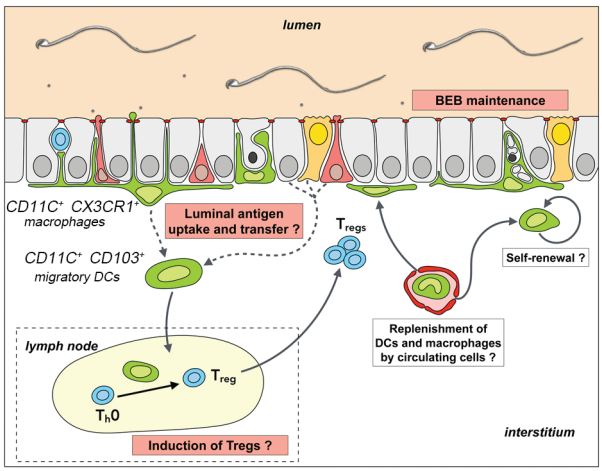

Figure 1: Localization, phenotype and possible functions of MPs in the steady state murine epididymis. The epididymal epithelium contains principal cells (gray), clear or narrow cells (orange), BCs (red), lymphocytes or halo cells (blue) and MPs (green). In this model, circulating Ly6Chi monocytes and DC precursors constitutively enter the epididymis and differentiate into mature CX3CR1$^+$ CD11c$^+$ F4/80$^+$ Mϕs, and CD11c$^+$ CD103$^+$ DCs, respectively. Resident Mϕs may also self-renew *in situ*. MPs could acquire luminal soluble antigens and/or particles directly via extension of intraepithelial processes, or indirectly using conduits created by principal, clear and BCs. Following antigen uptake, migratory CD103$^+$ DCs may migrate to lymph nodes to induce the development of FoxP3$^+$ regulatory T cells (Tregs) in order to regulate tolerance to luminal antigens, or interact locally with resident lymphocytes. In addition, MPs play a major role in the maintenance of the BEB by rapidly removing apoptotic epithelial cells and debris. DCs and Mϕs are also involved in the regulation of innate and adaptive immunity when a danger signal is sensed (infection, inflammation), and are also ideally positioned to survey circulating antigens. Our schematic diagram depicts observations made on the MP system in the IS; however, some MP functions might be segment specific. These putative MP functions in the steady state epididymis are largely inspired by studies of the gastrointestinal tract.[11,13,14,16,20] MPs: mononuclear phagocytes; BEB: blood-epididymis barrier; DC: dendritic cell; Mϕs: macrophages; BCs: basal cells; IS: initial segment.

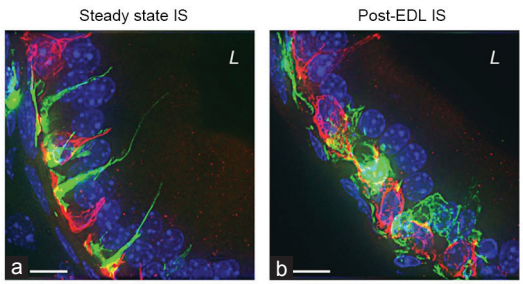

Figure 3: MP and BC response to the loss of luminal factors caused by EDL in the proximal mouse epididymis. In the steady state epididymis (**a**), CX3CR1$^+$ CD11c$^+$ intraepithelial Mϕs (green) and KRT5$^+$ BCs (red) sit in the basal region of the epithelium and occasionally project extensions (arrows) toward the luminal compartment. After 48 h EDL (**b**), MPs engulf apoptotic cells and debris and exhibit a phagocytic appearance, while BCs are not visibly involved in the apoptotic cell clearance process. Scale bars = 5 μm, L: lumen; MPs: mononuclear phagocytes; Mϕs: macrophages; BCs: basal cells; EDL: efferent duct ligation.

ducts and the vas deferens), and are also present in the interstitial space and the connective tissue capsule that covers the epididymis. Several studies have suggested that BCs of the rodent epididymis express Mϕ markers and exert an immunological function.[2,36,44,45] This long-standing confusion between epididymal BCs and intraepithelial

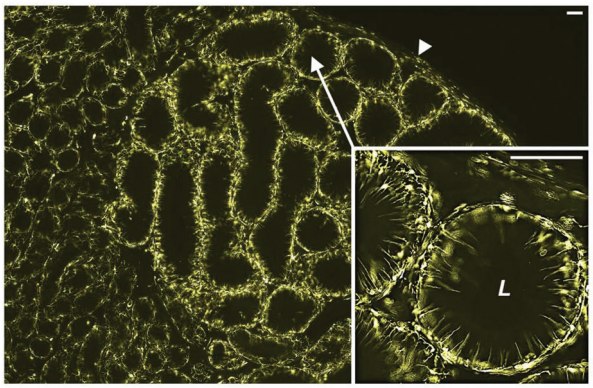

Figure 2: MPs in the proximal mouse epididymis. CD11c$^+$ and CX3CR1$^+$ MPs are abundant in the murine epididymis. This partial view of the most proximal region of a section of CD11c-EYFP mouse epididymis reveals CD11c$^+$ DCs and Mϕs. CD11c$^+$ MPs are present in the peritubular region as well as in the interstitium and the capsule (arrowhead) that wraps the epididymis. In the IS (brightest area), which is the most proximal epididymal segment, CD11c$^+$ (and CX3CR1$^+$) intraepithelial Mϕs extend dendritic processes toward the apical side of the epithelium, suggesting that this particular subset of MPs is in charge of directly surveying the luminal content, possibly in a segment-specific manner. Scale bars = 50 μm, L: lumen; MPs: mononuclear phagocytes; DCs: dendritic cell; Mϕs: macrophages; IS: initial segment.

Mϕs illustrates the importance of characterizing each subset of cell by capitalizing on tools and technologies recently introduced and newly discovered markers specific for each cell type. Indeed, BCs and Mϕs are both located in the basal region of the epithelium and share apparent morphological similarities. However, using powerful imaging techniques in combination with specific markers and flow cytometry-based analyses, Shum *et al.* have demonstrated that BCs and Mϕs represent two unequivocally distinct cell types.[5] Both occupy the basolateral compartment of the epididymal epithelium and, in the initial segment (IS), extend slender processes toward the lumen. Only intraepithelial Mϕs express F4/80, CX3CR1, and CD11c, while BCs express the widely acknowledged BC marker keratin5 (KRT5). Although BCs do not belong to the immune system, a contribution to local immunoregulatory mechanisms (possibly via crosstalk with neighboring MPs) cannot be excluded.

In the gut, one of the primary functions of epithelial Mϕs is to maintain the homeostasis of the epithelial barrier. The intestinal lumen is an extremely harsh environment; the thin epithelium is constantly challenged by trillions of microorganisms that make up the natural intestinal flora, pathogenic microbes, digestive acids and enzymes, as well as ingested materials that must be either assimilated or rejected. Comparatively, the epididymal luminal compartment may seem quiescent: during the entire reproductive life, the duct is mostly occupied by a complex yet stable fluid, radically changing just once with the introduction of spermatozoa at puberty. However, the physical integrity of the pseudostratified epithelium should constitute an essential prerequisite to the establishment of an immunoprotective environment for spermatozoa.

HOMEOSTASIS OF THE BLOOD-EPIDIDYMIS BARRIER

In addition to its immunological role, the BEB is an anatomical and physiological barrier that controls the exchange of molecules between the lumen and the interstitial space containing blood and lymph, thus allowing the formation of the specialized luminal environment in which sperm transit and mature.[31] Both immunoregulatory and physiological

roles of the BEB are strictly dependent on the proper maintenance of the single layer of epithelial cells that lines the entire duct. Over the past decade, work from many laboratories has revealed the crucial importance of apoptotic cell clearance in the maintenance of all epithelia. The rapid removal of defective epithelial cells is critical for maintaining both the physical integrity of epithelial barriers and the tolerance to autoantigens. Failed clearance of apoptotic cells can lead to the development of various pathogenic states, including autoimmune diseases.[52–55] Apoptotic cell clearance is a finely regulated multistep process that involves various molecular and cellular interactions, from the detection of apoptotic cells using "find-me" signals to their destruction. The professional effectors of phagocytic clearance are Mϕs and DCs, nevertheless neighboring epithelial cells may be involved in the process. Interestingly, apoptotic cells are rarely detected in the epididymal epithelium; however, MPs are conveniently abundant, suggesting the presence of highly efficient clearance mechanisms. In addition, intraepithelial Mϕs express CX3CR1, the receptor for the chemokine CX3CL1 (fractalkine), which has been identified as a find-me signal released by dying cells.

Efferent duct ligation (EDL) is a simple surgical procedure that provides an interesting opportunity to study apoptotic clearance in the epididymis; by preventing testicular factors from entering the IS, EDL induces a massive yet transient wave of epithelial apoptosis in the proximal epididymis.[45,56,57] Smith *et al.* have recently reported that epididymal intraepithelial CD11c$^+$ CX3CR1$^+$ Mϕs respond to EDL by rapidly engulfing apoptotic cells and debris, thus preserving the integrity of the apical tight junction network.[58] Contrasting with Mϕs, BCs do not seem to be involved in apoptotic cell clearance **(Figure 3)**; however, recent data have demonstrated that the absence of testis-derived luminal factors reduces their projections and furthermore, BCs could play a role in the post-EDL epithelial regeneration.[59] While the extreme situation caused by EDL is never encountered in physiological conditions, these results suggest that one of the primary functions of eMPs, and more specifically intraepithelial CX3CR1$^+$ CD11c$^+$ Mϕs, is to actively maintain the integrity of the BEB. However, Mϕs are much more than the janitors of the epithelium; by engulfing apoptotic cells, intraepithelial Mϕs could play an active role in the maintenance of peripheral tolerance to local self-antigens, including antigens displayed on or released by maturing spermatozoa.

EPIDIDYMAL MONONUCLEAR PHAGOCYTES AND TOLERANCE

In rodents as well as in humans, immunological tolerance is the result of fundamentally distinct mechanisms that involve both innate and adaptive immunity. Central tolerance is established in the bone marrow and thymus during the early stages of the immune system development. T and B cells that recognize self-antigens are removed or silenced, thus preventing the development of an immune response against the host's antigens. Germ cells and mature sperm constitute a rather unique challenge for the immune system; they start populating the reproductive system at puberty (long after the establishment of central tolerance), are abundant and express antigens that are not expressed elsewhere in the body. Accordingly, autoimmunity against sperm antigen is a direct cause of male infertility and underscores the fact that robust tolerogenic mechanisms must take place in the reproductive system during the entire reproductive life.[27,28] These mechanisms are known as peripheral tolerance,[60] which takes place after the initial maturation of B and T cells. Peripheral tolerance results from multiple complementary mechanisms that involve a variety of cellular and molecular actors, including MPs.

In the gut, CD103$^+$ DCs acquire antigens directly or indirectly (via epithelial cells or intraepithelial Mϕs), and then migrate to the MLNs via a CCR7-dependent mechanism.[13,14,16,24] Once in the MLN, they induce the development of a specialized subset of CD4$^+$ T cells known as regulatory T cells (Treg), primarily distinguishable by their expression of the transcription factor FoxP3. Induced Tregs exert a suppressor activity on effector T cells, thus preventing the development of an immune response against self or innocuous antigens. eMPs are ideally positioned to have a similar activity in the BEB, although experimental evidence remains to be provided. Strikingly similar to the small intestine, the proximal region of the epididymal duct contains CX3CR1$^+$ intraepithelial Mϕs that project numerous dendrites toward the luminal compartment. We have recently demonstrated that CX3CR1$^+$ CD11c$^+$ eMPs are potent phagocytes and occasionally penetrate the luminal compartment, suggesting that they have the ability to directly sample soluble antigens and particles from the lumen (Smith *et al.*,2014 *American Society of Andrology* meeting, Atlanta, USA). It is therefore tempting to speculate that some Mϕs may directly acquire material originating from spermatozoa in order to initiate a tolerogenic response, mediated by the CD11c$^+$ CD103$^+$ DCs that are also present in the epididymis.[1] Acquisition of sperm components might also occur via indirect mechanisms involving epithelial cells, or by modulating the permeability of apical tight junctions.[35] In addition, CD11c$^+$ MPs isolated from the whole murine epididymis have potent antigen-presenting capabilities *in vitro*.[1] The current challenge is to characterize the function of clearly defined subsets of Mϕs and DCs in the context of a complex epididymal architecture, where MPs are likely to have region-specific and possibly segment-specific activities. Importantly, despite their abundance and their heterogeneity, MPs represent only a few pieces in the immunological puzzle that is the epididymis. Several factors involved in immunosuppression have been identified in the epididymis, including interleukin-10, cyclo-oxygenase 1 and 2, transforming growth factor beta, and indoleamine 2,3-dioxygenase (*Ido*). *Ido* is an enzyme that exerts immunosuppressive effects by regulating the catabolism of tryptophan. In the gut, *Ido* is synthesized by CD11c$^+$ CD103$^+$ DCs and controls the development of Tregs, thus regulating oral tolerance.[61] The disruption of *Ido* in a mouse model modified the immunological equilibrium of the epididymis and, ultimately, impacted sperm quality and quantity.[62,63] While current data indicate that CD103$^+$ DCs are not the primary source of epididymal *Ido*, the influence of *Ido* on local DCs and Mϕs should be further investigated.

EPIDIDYMAL MONONUCLEAR PHAGOCYTES, CAPILLARIES AND LYMPHATIC VESSELS

The architecture of blood and lymphatic vasculature is expected to have a direct impact on eMP function by influencing their replenishment and migration toward LNs. Interestingly, several groups have observed that blood and lymphatic capillaries are mutually exclusive in the rodent epididymis: in the proximal regions, blood vessels and capillaries are extremely abundant, and lymphatic vessels are scarce. In contrast, the cauda contains a dense network of lymphatic vessels and few capillaries. In addition, capillaries from the IS are fenestrated and establish close interactions with the duct, thus facilitating a very active exchange of fluid, solutes and cells between the epididymal duct and the blood flow.[64–66] While the abundance of fenestrated capillaries in the IS might be predominantly related to the high rate of fluid reabsorption characteristic of this segment,[40,41] it should be noted that these capillaries are in close proximity with CX3CR1$^+$ Mϕs. Mucosal antigen sampling is not strictly unidirectional: in the gut, lamina propria CX3CR1$^+$ DCs closely interact with the fenestrated capillary network to facilitate surveillance of circulatory antigens, consequently

modulating the activation of mucosal T cells.[67] The strikingly similar network of vessels and MPs in the proximal epididymis leads us to believe that a subset of MPs might be in charge of surveying the blood compartment, thus creating another conduit in the BEB.

EPIDIDYMAL MONONUCLEAR PHAGOCYTES IN ACTIVE IMMUNE RESPONSES

The immunophysiology of the epididymis receives relatively little attention partly because this organ is not affected by major medical conditions. The most common pathology is epididymitis (inflammation of the epididymis), often in response to a bacterial infection of the urogenital tract. However, the immunological environment of the epididymis is also affected by other events, including aging, surgical procedures (such as vasectomy), physical trauma, as well as experimentally induced autoimmune disorders.[3,27,28,49,68] Vasectomy is often associated with the development of an autoimmune response against sperm antigens, as well the appearance of sperm granulomas in the epididymis.[46,49,69,70] Epididymal Mϕs and DCs are likely to be directly involved in the complex immune disturbance caused by vasectomy,[49] from the early sensing of danger signals in the distal epididymis to the late development of granulomas (masses of extravasated spermatozoa surrounded by immune cells) and sperm autoantibodies. It is worth noting that epididymal tumors are extremely rare.[71] We postulate that the dense network of MPs occupying the epididymis, including Mϕs that have been shown to be impressively efficient in the clearance of abnormal cells,[52,54,58] may play a role in the low incidence of epididymal cancer.

CONCLUSION

Despite an intense global effort to understand the immunophysiology of the intestinal mucosa over many decades, new information is continually revealed. Our discovery of a sophisticated network of DCs and Mϕs in the mouse epididymis has put the spotlight on one of the best-kept secrets of mammalian physiology, one that is challenging to study. Fortunately, the previously outlined similarities between these two organs, both functional and morphological, allow us to apply discoveries from the research effort in the intestine to postulate on the function of the epididymal mucosal immune system. Historically it has been accepted that the BEB physically separates the immune system from spermatozoa and the intricate and abundant blood vasculature unique to the IS of the epididymis unveils an "immunologically fragile site."[64] Contrarily, recent observations suggest that the IS may be a primary site for immunoregulation by virtue of a dense network of MPs projecting long intraepithelial dendrites exclusively in the IS. The IS lumen represents an opportunity for sperm leaving the testis to interact directly with the components of the epithelium, including intraepithelial MPs. In this respect, the IS might be seen as an "*in vivo* flow cytometer." Notwithstanding these findings and requiring extensive research, the IS is also a region that is comparatively poor in lymphatic vessels, which might suggest that intense trafficking of migratory DCs is not a primary feature of this segment. On the other hand, this might also suggest that the process of migration and antigen presentation is particularly potent, and a sparse network of lymphatic vessels is sufficient for maintaining immune homeostasis in this region. In the remaining segments, MPs line the basal region of the epithelium in addition to being scattered throughout the interstitium. Dendrites extending toward the lumen are not observed; however, thus suggesting that direct acquisition of luminal antigen does not occur, at least in the steady state epididymis, beyond the IS. Rather, any antigens acquired from the lumen must be shuttled through the epithelium. Interestingly, the lymphatic system is comparatively more generous in the distal epididymis, suggesting that either migration of APCs to LNs is not exclusive to the IS, or that the distal epididymis has a large requirement for clearing extravasated fluid. Speculation aside, the segmentation of the epididymal epithelium and the various morphologies and distribution of APCs must, therefore, be taken into consideration when exploring the function of this unique mucosa. Finally, if a germ cell "quality control" mechanism does take place in the epididymis as per previous suggestions,[62,63,72,73] eMPs are ideally positioned and equipped to play a role in the detection (and possible removal) of defective gametes in the IS.

Unraveling the mysteries of the epididymal mucosa will require an extensive basic research effort in favor of gaining a greater understanding of destructive inflammatory responses in the epididymis contributing to epididymitis and infertility, and ultimately exploiting the system in pursuit of an effective immunocontraceptive. Beyond the scope of reproductive immunology, the epididymis provides a clean site free of commensal bacteria that we anticipate will provide a fruitful tool for the broad study of mucosal immunology.

COMPETING INTERESTS

The author declares that they have no competing interests.

ACKNOWLEDGMENTS

Our research is supported by *National Institutes of Health* grant 5R01HD069623. We thank Claire R Barton for her help in proofing this manuscript.

REFERENCES

1 Da Silva N, Cortez-Retamozo V, Reinecker HC, Wildgruber M, Hill E, *et al*. A dense network of dendritic cells populates the murine epididymis. *Reproduction* 2011; 141: 653–63.
2 Mullen TE Jr, Kiessling RL, Kiessling AA. Tissue-specific populations of leukocytes in semen-producing organs of the normal, hemicastrated, and vasectomized mouse. *AIDS Res Hum Retroviruses* 2003; 19: 235–43.
3 Serre V, Robaire B. Distribution of immune cells in the epididymis of the aging Brown Norway rat is segment-specific and related to the luminal content. *Biol Reprod* 1999; 61: 705–14.
4 Serre V, Robaire B. Interactions of the immune system and the epididymis. In: Robaire B, Hinton BT, editors. The Epididymis: from Molecular to Clinical Practice. A Comprehensive Survey of the Efferent Ducts, the Epididymis and the Vas Deferens. New York: Kluwer Academic/Plenum; 2002. p. 219–31.
5 Shum WW, Smith TB, Cortez-Retamozo V, Grigoryeva LS, Roy JW, *et al*. Epithelial basal cells are distinct from dendritic cells and macrophages in the mouse epididymis. *Biol Reprod* 2014; 90: 90.
6 Geissmann F, Gordon S, Hume DA, Mowat AM, Randolph GJ. Unravelling mononuclear phagocyte heterogeneity. *Nat Rev Immunol* 2010; 10: 453–60.
7 Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, *et al*. Development of monocytes, macrophages, and dendritic cells. *Science* 2010; 327: 656–61.
8 Merad M, Sathe P, Helft J, Miller J, Mortha A. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu Rev Immunol* 2013; 31: 563–604.
9 Mildner A, Jung S. Development and function of dendritic cell subsets. *Immunity* 2014; 40: 642–56.
10 Hashimoto D, Miller J, Merad M. Dendritic cell and macrophage heterogeneity *in vivo*. *Immunity* 2011; 35: 323–35.
11 Cerovic V, Bain CC, Mowat AM, Milling SW. Intestinal macrophages and dendritic cells: what's the difference? *Trends Immunol* 2014; 35: 270–7.
12 Farache J, Zigmond E, Shakhar G, Jung S. Contributions of dendritic cells and macrophages to intestinal homeostasis and immune defense. *Immunol Cell Biol* 2013; 91: 232–9.
13 Mazzini E, Massimiliano L, Penna G, Rescigno M. Oral tolerance can be established via gap junction transfer of fed antigens from CX3CR1? Macrophages to CD103? Dendritic cells. *Immunity* 2014; 40: 248–61.
14 McDole JR, Wheeler LW, McDonald KG, Wang B, Konjufca V, *et al*. Goblet cells deliver luminal antigen to CD103$^+$ dendritic cells in the small intestine. *Nature* 2012; 483: 345–9.
15 Niess JH, Reinecker HC. Lamina propria dendritic cells in the physiology and pathology of the gastrointestinal tract. *Curr Opin Gastroenterol* 2005; 21: 687–91.
16 Niess JH, Reinecker HC. Dendritic cells: the commanders-in-chief of mucosal immune defenses. *Curr Opin Gastroenterol* 2006; 22: 354–60.

17 Niess JH, Reinecker HC. Dendritic cells in the recognition of intestinal microbiota. *Cell Microbiol* 2006; 8: 558–64.
18 Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, *et al.* A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* 2012; 336: 86–90.
19 Niess JH, Brand S, Gu X, Landsman L, Jung S, *et al.* CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* 2005; 307: 254–8.
20 Farache J, Koren I, Milo I, Gurevich I, Kim KW, *et al.* Luminal bacteria recruit CD103$^+$ dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation. *Immunity* 2013; 38: 581–95.
21 Steinman RM. Dendritic cells *in vivo*: a key target for a new vaccine science. *Immunity* 2008; 29: 319–24.
22 Steinman RM, Banchereau J. Taking dendritic cells into medicine. *Nature* 2007; 449: 419–26.
23 Savina A, Amigorena S. Phagocytosis and antigen presentation in dendritic cells. *Immunol Rev* 2007; 219: 143–56.
24 Scott CL, Aumeunier AM, Mowat AM. Intestinal CD103$^+$ dendritic cells: master regulators of tolerance? *Trends Immunol* 2011; 32: 412–9.
25 Cader MZ, Kaser A. Recent advances in inflammatory bowel disease: mucosal immune cells in intestinal inflammation. *Gut* 2013; 62: 1653–64.
26 Hinton B, Turner T. Is the epididymis a kidney analogue? *Physiology* 1988; 3: 28–31.
27 Hedger MP. Immunophysiology and pathology of inflammation in the testis and epididymis. *J Androl* 2011; 32: 625–40.
28 Hedger MP, Hales DB. Immunophysiology of the male reproductive tract. In: Neill JD, editor. Knobil and Neill's Physiology of Reproduction.3rd ed. New York: Elsevier Academic Press; 2006. p. 1195–286.
29 Hoffer AP, Hinton BT. Morphological evidence for a blood-epididymis barrier and the effects of gossypol on its integrity. *Biol Reprod* 1984; 30: 991–1004.
30 França LR, Auharek SA, Hess RA, Dufour JM, Hinton BT. Blood-tissue barriers: morphofunctional and immunological aspects of the blood-testis and blood-epididymal barriers. *Adv Exp Med Biol* 2012; 763: 237–59.
31 Mital P, Hinton BT, Dufour JM. The blood-testis and blood-epididymis barriers are more than just their tight junctions. *Biol Reprod* 2011; 84: 851–8.
32 Dubé E, Cyr DG. The blood-epididymis barrier and human male fertility. *Adv Exp Med Biol* 2012; 763: 218–36.
33 Fijak M, Meinhardt A. The testis in immune privilege. *Immunol Rev* 2006;213: 66–81.
34 Meinhardt A, Hedger MP. Immunological, paracrine and endocrine aspects of testicular immune privilege. *Mol Cell Endocrinol* 2011; 335: 60–8.
35 Stammler A, Müller D, Tabuchi Y, Konrad L, Middendorff R. TGFßs modulate permeability of the blood-epididymis barrier in an *in vitro* model. PLoS One 2013; 8: e80611.
36 Arrighi S. Are the basal cells of the mammalian epididymis still an enigma? *Reprod Fertil Dev* 2014; 26: 1061–71.
37 Belleannée C, Thimon V, Sullivan R. Region-specific gene expression in the epididymis. *Cell Tissue Res* 2012; 349: 717–31.
38 Cornwall GA. New insights into epididymal biology and function. *Hum Reprod Update* 2009; 15: 213–27.
39 Shum WW, Ruan YC, Da Silva N, Breton S. Establishment of cell-cell cross talk in the epididymis: control of luminal acidification. *J Androl* 2011; 32: 576–86.
40 Robaire B, Hinton BT, Orgebin-Crist MC. The epididymis. In: Neill JD, editor. Knobil and Neill's Physiology of Reproduction. 3rd ed. New York: Elsevier Academic Press; 2006. p. 1072–148.
41 Robaire B, Hermo L. Efferent ducts, epididymis, and vas deferens: structure, functions, and their regulation. In: Knobil E, Neil J, editors. The Physiology of Reproduction. New York: Raven Press; 1988.
42 Flickinger CJ, Bush LA, Howards SS, Herr JC. Distribution of leukocytes in the epithelium and interstitium of four regions of the Lewis rat epididymis. *Anat Rec* 1997; 248: 380–90.
43 Pöllänen P, Cooper TG. Immunology of the testicular excurrent ducts. *J Reprod Immunol* 1994; 26: 167–216.
44 Yeung CH, Nashan D, Sorg C, Oberpenning F, Schulze H, *et al.* Basal cells of the human epididymis – antigenic and ultrastructural similarities to tissue-fixed macrophages. *Biol Reprod* 1994; 50: 917–26.
45 Seiler P, Cooper TG, Yeung CH, Nieschlag E. Regional variation in macrophage antigen expression by murine epididymal basal cells and their regulation by testicular factors. *J Androl* 1999; 20: 738–46.
46 Flickinger CJ, Howards SS, Herr JC. Effects of vasectomy on the epididymis. *Microsc Res Tech* 1995; 30: 82–100.
47 Beagley KW, Wu ZL, Pomering M, Jones RC. Immune responses in the epididymis: implications for immunocontraception. *J Reprod Fertil Suppl* 1998; 53: 235–45.
48 Itoh M, Terayama H, Naito M, Ogawa Y, Tainosho S. Tissue microcircumstances for leukocytic infiltration into the testis and epididymis in mice. *J Reprod Immunol* 2005; 67: 57–67.
49 Wheeler K, Tardif S, Rival C, Luu B, Bui E, *et al.* Regulatory T cells control tolerogenic versus autoimmune response to sperm in vasectomy. *Proc Natl Acad Sci U S A* 2011; 108: 7511–6.
50 Lindquist RL, Shakhar G, Dudziak D, Wardemann H, Eisenreich T, *et al.* Visualizing dendritic cell networks *in vivo*. *Nat Immunol* 2004; 5: 1243–50.
51 Jung S, Aliberti J, Graemmel P, Sunshine MJ, Kreutzberg GW, *et al.* Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Mol Cell Biol* 2000; 20: 4106–14.
52 Elliott MR, Ravichandran KS. Clearance of apoptotic cells: implications in health and disease. *J Cell Biol* 2010; 189: 1059–70.
53 Elliott MR, Ravichandran KS. ELMO1 signaling in apoptotic germ cell clearance and spermatogenesis. *Ann N Y Acad Sci* 2010; 1209: 30–6.
54 Hochreiter-Hufford A, Ravichandran KS. Clearing the dead: apoptotic cell sensing, recognition, engulfment, and digestion. *Cold Spring Harb Perspect Biol* 2013; 5: a008748.
55 Ravishankar B, McGaha TL. O death where is thy sting? Immunologic tolerance to apoptotic self. *Cell Mol Life Sci* 2013; 70: 3571–89.
56 Turner TT, Johnston DS, Finger JN, Jelinsky SA. Differential gene expression among the proximal segments of the rat epididymis is lost after efferent duct ligation. *Biol Reprod* 2007; 77: 165–71.
57 Turner TT, Riley TA. p53 independent, region-specific epithelial apoptosis is induced in the rat epididymis by deprivation of luminal factors. *Mol Reprod Dev* 1999; 53: 188–97.
58 Smith TB, Cortez-Retamozo V, Grigoryeva LS, Hill E, Pittet MJ, *et al.* Mononuclear phagocytes rapidly clear apoptotic epithelial cells in the proximal epididymis. *Andrology* 2014; 2: 755–62.
59 Kim B, Roy J, Shum WW, Da Silva N, Breton S. Role of testicular luminal factors on Basal cell elongation and proliferation in the mouse epididymis. *Biol Reprod* 2015; 92: 9.
60 Mueller DL. Mechanisms maintaining peripheral tolerance. *Nat Immunol* 2010; 11: 21–7.
61 Matteoli G, Mazzini E, Iliev ID, Mileti E, Fallarino F, *et al.* Gut CD103$^+$ dendritic cells express indoleamine 2,3-dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction. *Gut* 2010; 59: 595–604.
62 Guiton R, Henry-Berger J, Drevet JR. The immunobiology of the mammalian epididymis: the black box is now open! Basic Clin Androl. 2013 Oct 2;23:8. doi: 10.1186/2051-4190-23-8. eCollection 2013.
63 Jrad-Lamine A, Henry-Berger J, Damon-Soubeyrand C, Saez F, Kocer A, *et al.* Indoleamine 2,3-dioxygenase1 (ido1) is involved in the control of mouse caput epididymis immune environment. *PLoS One* 2013; 8: e66494.
64 Hirai S, Naito M, Terayama H, Ning Q, Miura M, *et al.* Difference in abundance of blood and lymphatic capillaries in the murine epididymis. *Med Mol Morphol* 2010; 43: 37–42.
65 Abe K, Takano H, Ito T. Microvasculature of the mouse epididymis, with special reference to fenestrated capillaries localized in the initial segment. *Anat Rec* 1984; 209: 209–18.
66 Suzuki F. Microvasculature of the mouse testis and excurrent duct system. *Am J Anat* 1982; 163: 309–25.
67 Chang SY, Song JH, Guleng B, Cotoner CA, Arihiro S, *et al.* Circulatory antigen processing by mucosal dendritic cells controls CD8(+) T cell activation. *Immunity* 2013; 38: 153–65.
68 Rival C, Wheeler K, Jeffrey S, Qiao H, Luu B, *et al.* Regulatory T cells and vasectomy. *J Reprod Immunol* 2013; 100: 66–75.
69 Adams CE, Wald M. Risks and complications of vasectomy. *Urol Clin North Am* 2009; 36: 331–6.
70 Flickinger CJ, Howards SS, Bush LA, Baker LA, Herr JC. Antisperm autoantibody responses to vasectomy and vasovasostomy in Fischer and Lewis rats. *J Reprod Immunol* 1995; 28: 137–57.
71 Yeung CH, Wang K, Cooper TG. Why are epididymal tumours so rare? *Asian J Androl* 2012; 14: 465–75.
72 Sutovsky P. Ubiquitin-dependent proteolysis in mammalian spermatogenesis, fertilization, and sperm quality control: killing three birds with one stone. *Microsc Res Tech* 2003; 61: 88–102.
73 Sutovsky P, Neuber E, Schatten G. Ubiquitin-dependent sperm quality control mechanism recognizes spermatozoa with DNA defects as revealed by dual ubiquitin-TUNEL assay. *Mol Reprod Dev* 2002; 61: 406–13.