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Oxidative stress, spermatozoa and leukocytic infiltration: relationships forged by the opposing forces of microbial invasion and the search for perfection

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ABSTRACT

This review addresses the complex relationships that exist between spermatozoa and the immune system and highlights the role of oxidative stress in regulating the direction and functional relevance of these interactions. Spermatozoa are potentially antigenic; however, in the testes and epididymis these cells are sequestered behind physical barriers and benefit from a tolerogenic state generated through the mediation of indoleamine dioxygenase. In the female there are no such barriers; however, inseminated spermatozoa are protected by the concomitant presence of seminal plasma. The latter possesses immunosuppressive properties, a powerful array of antioxidants and cytokines that modulate the immunological response to semen deposition. Subsequent to insemination, leukocytic infiltration of the female tract occurs to facilitate the removal of millions of residual moribund and senescent spermatozoa, while allowing the most competent cells to ascend to the site of fertilization. The post-insemination phagocytosis of non-viable spermatozoa is 'silent' in the sense that no reactive oxygen species (ROS) or pro-inflammatory cytokines are generated. The silent phagocytosis of senescent spermatozoa is a response to markers, such as phosphatidylserine, which are expressed on the surface of spermatozoa as they engage in the intrinsic apoptotic cascade. By contrast, infection can bring fully activated leukocytes into the male reproductive tract that are actively generating ROS and releasing pro-inflammatory cytokines. Such free-radical-generating leukocytes have the potential to seriously damage the functionality of spermatozoa as well as the integrity of their DNA, particularly in vitro, when these cells are devoid of the antioxidant protection afforded by seminal plasma.

1. Introduction – the unique status of spermatozoa

The mechanisms by which spermatozoa interact with the immune system have been a matter of deliberation and controversy for many years. Spermatozoa are potentially antigenic cells that under the wrong circumstances can elicit the formation of anti-sperm antibodies in both the male and female reproductive tracts (Chiu and Chamley, 2004). Furthermore, the presence of such antibodies has been linked with a wide range of adverse reproductive outcomes (Jones, 1994) as a result of such processes as complement-mediated sperm immobilization, impaired sperm transport through the endocervical canal, disrupted sperm–egg interaction, sperm agglutination and impaired embryo survival secondary to the presence of epitopes that are shared by spermatozoa and trophoblast tissue (Choudhury and Knapp, 2001). In light of such factors, it is not surprising that differentiating male germ cells are

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physically separated from the immune system behind the blood–testes barrier. Any disruption of this barrier as a result of physical trauma or infection rapidly leads to the precipitation of an immune response and in some species, such as the guinea pig, to a profound orchitis (Meinhardt and Hedger, 2011). Sertoli cells are not only instrumental in creating the blood–testes barrier, but also maintain a tolerogenic state towards the germ line through the activity of indoleamine dioxygenase (IDO), as a consequence of the latter's ability to regulate the bioavailability of tryptophan as well as the capacity of tryptophan metabolites to suppress T-cell-mediated immune responses (Fallarino et al., 2009).

During epididymal transit the spermatozoa are again protected by a physical barrier laid down by the epididymal epithelium, which displays a complex array of apical junctional complexes to reinforce its physical integrity (França et al., 2012). In addition, the epididymis is programmed to exhibit a tolerogenic state towards spermatozoa by IDO, in a similar manner to the testes (Dai and Zhu, 2010). This enzyme is present in extremely high concentrations in the caput epididymis and appears to be a key player in not only maintaining a tolerogenic state in this organ, but also in regulating the quality of gametes emerging into the vas deferens. Specifically, functional deletion of IDO results in a statistically significant increase in sperm cell numbers in the epididymis in concert with a dramatic reduction of sperm quality, as judged by their morphology (Jrad-Lamine et al., 2011). These results suggest the existence of an active quality control mechanism operating within the caput epididymis that serves to ensure that only the fittest, most functional of cells are allowed to contribute to the ejaculate. The mediators of such an epididymal quality control mechanism are probably dendritic cells that project long cytoplasmic processes between caput epithelial cells towards the lumen. These dendritic cells express leucocyte markers and are strategically positioned to contribute to a remarkable process that allows millions of potentially immunogenic spermatozoa to pass through the epididymal lumen, while permitting selection and removal of any defective cells that are present (Da Silva et al., 2011).

While achieving this complex balance of tolerance towards sperm-specific antigens and intolerance towards defective gametes, the epididymis is also endowed with many features of the innate immune system to protect the transiting spermatozoa from the consequences of infection. Thus, the epididymis is known to elaborate antimicrobial defensins (Yamaguchi et al., 2002; Com et al., 2003), to express toll-like receptors (Palladino et al., 2007) and to be capable of generating pro-inflammatory cytokines such as tumour necrosis factor and interleukin 1 (Zhao et al., 2008). Furthermore, leukocytes including T-cells and macrophages can also be found in the epididymal interstitium following infection. Interestingly, however, these cells are rarely seen entering the epididymal lumen (Nashan et al., 1993). In this context, the abundant presence of macrophage migration inhibitory factor (MIF) in the epididymis may be responsible for regulating the intraluminal migration of macrophages in order to avoid inadvertent contact between the immune system and spermatozoa (Eickhoff et al., 2006).

After ejaculation, a completely different kind of tolerance is established in the female reproductive tract. In this case, the spermatozoa are not shielded from the maternal immune system. On the contrary, there is a massive leukocytic infiltration into the cervix and uterus following insemination that serves to remove the millions of residual spermatozoa littering the female reproductive tract, while still allowing the highest quality cells to ascend to the site of fertilization. The interaction between spermatozoa and the female immune system is therefore extremely sophisticated and complex, involving elements of immune tolerance and sperm selection on the one hand, while stimulating leukocytic infiltration and the phagocytosis of senescent cells on the other. Furthermore, the phagocytosis of spermatozoa in both the male and the female reproductive tract can be characterized as *silent* in the sense that no reactive oxygen species (ROS) or pro-inflammatory cytokines are produced (D'Cruz et al., 1992; Rossi and Aitken, 1997).

The mechanisms that underpin these different facets of sperm–leucocyte interaction in the male and female reproductive tract form the subject matter of this review. We shall examine the biological nature of these interactions and consider the central role that oxidative stress plays in their regulation, beginning with the role of apoptosis in shaping the way in which spermatozoa control their interaction with phagocytic leukocytes.

2. Apoptosis in spermatozoa and oxidative DNA damage

Spermatozoa are characterized by an intrinsic tendency to default to a truncated form of apoptosis if they experience stress following spermiation (Koppers et al., 2011). This apoptotic pathway begins with the stimulation of ROS generation by the mitochondria and culminates in cell death (Fig. 1). The pathways that can initiate such a response from human spermatozoa are numerous. They may involve local or systemic antioxidant depletion (Aitken, 1995; Gharagozloo and Aitken, 2011), exposure to radiofrequency electromagnetic radiation or heat (De Iuliis et al., 2009a), exposure to heavy metals such as cadmium (Xu et al., 2003), prolonged culture in vitro (Muratori et al., 2003), low ejaculation frequency as a consequence of spinal cord injury (Zhang et al., 2013), reproductive toxicants of various kinds (Aitken and Curry, 2011; Barratt et al., 2010), impaired spermiogenesis resulting in retention of excess residual cytoplasm (Weng et al., 2002; Gomez et al., 1996) and poor chromatin compaction (De Iuliis et al., 2009b). The net effect of any one or more of these stressful situations is to initiate a form of regulated senescence in these cells with attributes similar to (but not identical to) the intrinsic apoptotic cascade.

The trigger for this truncated apoptotic cascade in human spermatozoa is a failure to fully maintain the phosphorylation status of phosphatidylinositol 3-kinase (PI3-kinase) and RAC-alpha serine/threonine-protein kinase (AKT1) (Koppers et al., 2011). This pathway appears to be critical for the maintenance of sperm survival by virtue of its ability to prevent these cells from defaulting to an apoptotic state, via mechanisms involving the