Sperm Interact with Human Cervical Epithelial Cells to Modulate Cytokine Synthesis

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Seminal fluid introduced into the female reproductive tract at coitus induces an inflammation-like response within the cervical tissues of women. The response involves induction of cytokines and chemokines to drive recruitment and activation of leukocytes that in turn selectively edit the sperm population able to access oocytes, and promote immune tolerance towards male alloantigens to potentially facilitate embryo implantation. Soluble factors in seminal plasma are important mediators of seminal fluid signalling. Recently we showed in mice that sperm also facilitate the female response – but whether sperm contribute to the cervical immune response in humans is unclear. In this study, we evaluated the hypothesis that human sperm interact with Ect1 ectocervical epithelial cells to induce cytokine production and alter gene expression *in vitro*. Ten normozoospermic men (according to WHO VI guidelines) of reproductive age provided semen samples for the study. Ect1 cells were incubated with 10% (v/v) whole semen, 10% seminal plasma, 10% washed sperm, or washed sperm at fixed concentrations of 1, 5, and 10 x 10^6 sperm/mL, then gene expression and cytokine secretion were assessed in 8- and 24-hour supernatants by TaqMan Human inflammation panel OpenArray® and multiplex microbead assay, respectively. Scanning and transmission electron microscopy was used to visualize physical interactions between sperm and Ect1 cells. Washed sperm (10% v/v equivalent), as well as whole semen (10%) and seminal plasma (10%), all induced Ect1 cell production of CSF1, CXCL8, and CXCL11, whilst only semen and seminal plasma induced production of CSF2, CSF3, IL1B, IL6, IL20, CXCL1, CXCL16, CCL3, and CCL20 (n=10, all $P < 0.05$). Sperm-mediated induction occurred in a dose-dependent manner with the highest dose (10 x 10^6 sperm/mL) inducing maximal increase in Ect1 cell production of CSF2, CSF3, IL1A, CXCL8, CXCL11, CXCL16, CCL3 (n=10, all $P < 0.05$). IL1Ra production was inhibited by both whole semen and sperm, whilst CXCL10 was inhibited by whole semen and seminal plasma (n=10, $P < 0.05$). Gene expression analysis revealed a total of 178 genes as differentially regulated by treatment with seminal fluid components, with 128 genes upregulated and 50 genes downregulated (n=6, $P < 0.05$). Many cytokine and chemokine genes were upregulated after contact with washed sperm, as well as whole semen and seminal plasma, with CSF2, IL6, IL33, CXCL2, CXCL3, and CXCL8 all amongst the top 20 most upregulated genes across all three treatments. Principal component analysis identified different expression patterns induced by washed sperm compared to the similar expression patterns elicited by whole semen and seminal plasma. Electron microscopy showed evidence of sperm head attachment to microvilli on the Ect1 cell surface, and in some instances, complete engulfment by Ect1 cells. Collectively this data shows that human sperm have capacity to interact closely with cervical epithelial cells and can act to modulate their cytokine production and gene expression profile. Understanding the mechanisms that mediate this

interaction will progress understanding of biological processes by which maternal immune tolerance is generated and may aid in the development of interventions that improve immune receptivity to embryo implantation.