Seminal plasma regulation of the post-coital inflammatory response in the human cervix

David James Sharkey

Research Centre for Reproductive Health,
Department of Obstetrics and Gynaecology,
University of Adelaide, Adelaide,
Australia

A thesis submitted to the University of Adelaide in fulfilment of the requirements for admission to the degree Doctor of Philosophy

August 2005

Abstract

In mice and other mammalian species, deposition of semen into the female reproductive tract elicits a local inflammatory response. Whether a comparable response occurs within the human cervix has not previously been studied. The experiments described in this thesis demonstrate, using cervical tissue biopsies taken before and after intercourse, that exposure to semen elicits an infiltration of leukocytes into the cervical tissue of peri-ovulatory women. Immunohistochemical analysis identified macrophages and dendritic cells as the predominant leukocytes recruited into the cervical epithelium and stroma following intercourse. Cytotoxic / suppressor T lymphocytes and memory T cells were also increased. Comparable responses were not detected following condom-protected intercourse. Quantitative real-time PCR was performed on duplicate tissue biopsies to investigate the molecular regulation of this response. Expression of GM-CSF, a potent stimulator of myeloid cell recruitment, was found to increase by 2.5-fold following unprotected intercourse. Trends towards increased IL-6 and IL-8 mRNA were also observed. Condom-protected intercourse did not activate cytokine expression, further suggesting that exposure to semen, as opposed to mechanical trauma, provides the inflammatory stimulus.

In an in vitro model using the immortalised Ect-1 cell line, TGFβ was identified as a candidate active seminal factor. All three TGFβ isoforms were capable of mimicking the stimulatory ability of seminal plasma in Ect-1 cells and were comparable in their capacity to stimulate both GM-CSF and IL-6 expression in a dose-responsive manner. The addition of TGFβ isoform-specific neutralising antibodies inhibited seminal plasma-induced increases in these cytokines. However TGFβ was unable to stimulate IL-8 production. Addition of IFNγ was found to strongly inhibit TGFβ-stimulated GM-CSF production, and 19-OH PGE_1 was found to increase IL-6 and IL-8, but not GM-CSF production. Responses to seminal plasma constituents were almost exactly replicated in primary cultures of human ectocervical cells. These results identify TGFβ as the major active constituent in human seminal plasma and indicate that other seminal agents, 19-OH PGE_1 and IFNγ, interact with TGFβ to differentially regulate cervical cytokine expression.

Finally, whether human seminal plasma cytokine content was associated with fertility in men was examined. No relationship between seminal plasma $TGF\beta_1$, $TGF\beta_2$, $TGF\beta_3$, IL-8 or bacterial endotoxin content and fertility status was observed. However, there was an increased likelihood of high IFNγ

content in the male partners of couples experiencing infertility, most notable in recurrent miscarriage. The discriminating value of IFNγ was increased when evaluated as a ratio of total TGFβ content.

Inflammatory changes after exposure of the female reproductive tract to seminal plasma are implicated in 'conditioning' the maternal immune response, to facilitate successful embryo implantation and pregnancy. The studies described in this thesis provide a mechanistic basis for the observations linking exposure to semen with pregnancy success in humans and have expanded our knowledge of the cellular and molecular events that occur within the female reproductive tract following intercourse. Seminal plasma can therefore no longer be thought of as merely a transport medium for spermatozoa, rather as a means for communication between the male and female reproductive tissues, potentially required for optimal pregnancy success.