INDUCTION OF MITOGENESIS AND CELL-CELL ADHESION BY PORCINE SEMINAL PLASMA

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ABSTRACT

The study described in this thesis seeks to evaluate the nature of the interactions occurring between semen and cells of the uterus that occur following mating in pig. This thesis describes a previously unrecognized and novel ability of porcine seminal plasma to induce dose dependent cell-cell adhesion and mitogenesis amongst peripheral blood lymphocytes *in vitro*. The induction of cell-cell adhesion was shown to occur independently of the induction of mitogenesis and was not associated with immunosuppression or cytotoxicity. Seminal plasma induced homotypic cell-cell adhesion amongst T cells and macrophage/monocytes, but not amongst B cells. The response was inhibited by the exogenous fibronectin tetrapeptide, arg-gly-asp-ser (RGDS), inhibitors of intracellular signalling, cytochalasins, heparin and required divalent metal ions. This evidence indicated that the mechanism of cell-cell adhesion was analogous to that occurring between regulated cell surface integrins and molecules of the extracellular matrix and was the result of cellular activation by seminal factors. Integrins are ubiquitous cell surface heterodimeric glycoproteins, consisting of α and β subunits linked to the cytoskeleton via transmembrane linkages. Furthermore, they appear on all cell types and extend throughout the most of the phylogentic tree.

Assaying the various boar accessory gland secretions on lymphocytes *in vitro*, indicated that the mitogenic and adhesive activity originated in the seminal vesicles. Furthermore, surgical removal of the seminal vesicles resulted in ejaculates devoid of mitogenic and cell-cell adhesive activity. Fractions from chromatographic separations of seminal vesicle proteins produced by cation exchange, hydrophobic interaction, diafiltration, Phenyl-Superose™ and C-18 separations, were assayed for induction of cell-cell adhesion amongst lymphocytes *in vitro*. Reversed phase separations

conducted on Phenyl-Superose, revealed two distinct forms of cell-cell adhesion; one forming a network of inter-connecting strands and the other forming regular circular clumps of adhered cells. Reversed phased chromatography of the former on C-18 silica revealed a single peak of activity containing a protein of 15kDa molecular weight, which represented approximately 0.5-1% of the total seminal vesicle fluid protein content. Purity of the adhesion inducing factor-1 (AIF-1), was estimated at 97% by N-terminal sequencing, from which a 32 amino acid sequence was determined. Screening of the SwissProt protein sequence data base revealed that AIF-1 is a novel protein. A region of the N-terminal amino acid sequence of AIF-1 is homologous to a highly conserved region in two bovine seminal vesicle glycoproteins, of similar molecular weight to AIF-1. This sequence also appeared in the collagen binding domain of type II bovine fibronectin. The bovine seminal vesicle proteins share a high degree of homology with the collagen binding domain of type II bovine fibronectin and together with AIF-1, potentially represent a novel class of bioactive proteins with unique biological activites. Northern analysis of total porcine RNA by a synthetic 51mer DNA oligonucleotide complementary to the predicted mRNA identified an abundant mRNA species of 0.9kb.

The *in vivo* role of seminal plasma proteins, which functionally upregulate cell surface integrins remains uncertain. However, cellular adhesive interactions mediated through integrins, particularly in leukocytes, transduce a variety of intracellular signals important to the regulation of growth, development, differentiation, gene expression and activation state of the cell. Furthermore, expression of functionally upregulated cell surface adhesion receptors is a feature of cellular activation, particularly at sites of inflammation. Hence, the modulation of function and activation of uterine leukocytes and possibly of other cell types within the uterus by seminal plasma following mating is postulated.

Studies in rodents indicate that seminal plasma induces a post-mating uterine inflammatory response, associated with cytokine production by lymphoid and epithelial cells. Uterine and embryo derived cytokines are recognized as playing a role in the regulation of embryonic and uterine development during early pregnancy. In this regard, seminal plasma adhesion inducing proteins such as AIF-1, may augment the post-mating uterine inflammatory response and thus, participate in the esablishment of an appropriate uterine millieu important to the establishment of pregnancy.

The mechanisms involved in the regulation of cell adhesive interactions and its cellular consequences, remains one of the most fundamentally important and potentially rewarding aspects of cell biology.