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# The relationship between immunosuppressive activity and immunoregulatory cytokines in seminal plasma: Influence of sperm autoimmunity and seminal leukocytes

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**Abstract**

While the contributions of prostasomes, polyamines and prostaglandins to the immunosuppressive activity (ISA) of human seminal plasma have been well-characterised, the contribution of immunoregulatory cytokines found in seminal plasma has received relatively little attention. Semen samples were collected from adult men displaying normospermic parameters, sperm antibodies or substantially elevated seminal leukocytes. Samples were processed through ultracentrifugation and dialysis (<3500 Da) to remove prostasomes, polyamines and prostaglandins, and then assayed for ISA by an in vitro T lymphocyte inhibition assay, as well as by specific immunoassays for transforming growth factor $\beta_1$ (TGF$\beta_1$), interleukin-10 (IL-10), activin A and the activin-binding protein, follistatin. Seminal plasma from all groups retained substantial ISA following processing. Compared with normospermic men, this 'large' molecular weight ISA fraction was significantly increased in a subset of men with sperm antibodies, but was not altered in the group with elevated leukocytes. There was no relationship between ISA and any cytokine examined, and only TGF$\beta_1$ was present at levels sufficient to contribute to ISA. Inhibition with a TGFβ-specific antibody reduced ISA in seminal plasma by approximately 50%. Across all patients, TGF$\beta_1$ levels were positively correlated with sperm numbers in the ejaculate

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and with activin A, but not with follistatin or IL-10. Activin A and IL-10 also displayed a positive relationship, and elevated leukocytes was associated with a significant elevation of IL-10 and activin A, but not TGFβ$_1$. It is concluded that 'large' molecular weight molecules, the most important of which appears to be TGFβ$_1$, make a significant contribution to immunosuppression by human seminal plasma.


*Keywords:* Activin; Autoimmunity; Assays; Inflammation; Interleukin-10; Transforming growth factor β

## 1. Introduction

The inhibitory effect of seminal plasma in T lymphocyte functional assays in vitro has been attributed to a number of factors, including prostasomes (Kelly et al., 1991), oxidised polyamines (Allen and Roberts, 1986), prostaglandins of the E series (Skibinski et al., 1992), non-specific lymphocyte-suppressing proteins (Maccioni et al., 2001; Veselský et al., 2002) and immunoregulatory cytokines (Nocera and Chu, 1993; Rajasekaran et al., 1996). In particular, prostaglandins and cytokines are well-characterised regulators of immunity and inflammation, and are most likely to play a significant immunological role in the male and female reproductive tracts. With respect to immunosuppression by seminal plasma, the prostaglandins have received the most study, with less attention paid to the contribution of immunoregulatory cytokines. Cytokines with immunosuppressive activity that have been positively identified in human seminal plasma are transforming growth factor β$_1$ (TGFβ$_1$) and TGFβ$_2$ (Nocera and Chu, 1993; Srivastava et al., 1996; Loras et al., 1999), interleukin-10 (IL-10) (Rajasekaran et al., 1996; Huleihel et al., 1999; Miller et al., 2002) and activin A (Anderson et al., 1998). While a number of studies have investigated changes in the levels of these particular cytokines in various infertility conditions, their responses to immune events within the male reproductive tract have not been investigated.

In general, infection and inflammation lead to up-regulation of cytokines, which contributes to activation of either type 1 (cell-mediated) and type 2 (antibody-mediated) responses of the immune system (Jankovic et al., 2001). The cytokines of the type 2 response, particularly IL-10, and specific immunosuppressive cytokines such as TGFβ$_1$ also play an important role in limiting and ultimately resolving the immune response (MacDonald, 1998). In addition to resolution of the inflammatory/immune response, production of IL-10 and the TGFβs is generally associated with protection against autoimmune disease (Letterio and Roberts, 1998; Volk et al., 2001). Activin A is a member of the TGFβ family which inhibits both T and B lymphocyte activity in vitro and opposes the action of the key inflammatory cytokines IL-1 and IL-6 (Phillips et al., 2001). In contrast to TGFβ, which is regulated by processing of a biologically inactive (latent) precursor to a mature (active) form, the biological activity of activin A in vivo is regulated by a specific binding protein called follistatin (Phillips et al., 2001). It is likely that immune events within the male reproductive tract are associated with alterations in these cytokines and/or their bio-available levels. In support of this concept we have previously shown that, following removal of prostasomes and inactivation of polyamine activity, there is an inverse relationship between T lympho-