Effect of intra-rectal administration of boar seminal immunosuppressive fraction on mouse lymphocytes

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The repeated deposition of an immunosuppressive fraction isolated from boar vesicular gland secretion into the rectum of healthy male and female mice reduced responses of lymphocytes to mitogens *in vitro*. Rectal deposition of the immunosuppressive component also led to a decrease in the activity of plaque-forming cells. These findings indicate that repeated rectal deposition of semen may compromise some aspects of the immune system and may be an important cofactor in the development of viral or bacterial infections among homosexual men.

Introduction

Seminal plasma contains components that can suppress immune responses (Quayle and James, 1990) and syngeneic mouse spermatozoa injected *i.v.* are immunosuppressive as determined by mixed lymphocyte culture (Hurtenbach and Shearer, 1982). Spermatozoa are autoantigenic for males and isoantigenic for females. However, adults do not exhibit sensitization to spermatozoa.

The emergence of the acquired immune deficiency syndrome (AIDS) prompted us to investigate the immunosuppressive components in reproductive tract secretions. In homosexual men, the highest risk group for AIDS, immunological abnormalities are often observed without clinical signs of the disease (Krohn *et al.*, 1985; McCune, 1991). These findings suggest that the infectivity or activity of AIDS-associated virus might depend on the immunity of the host. Seminal plasma inhibits the activity of most cells of the immune system and interferes with the generation and activity of some soluble components involved in the natural defence against infections (Emoto *et al.*, 1990; Quayle *et al.*, 1989). Semen deposition in the gut may play a role in the development of viral infections among homosexual men (Shearer and Rabson, 1984; Olsen and Shields, 1984).

Suppressive activity of seminal plasma *in vitro* can be assigned to spermine (Shohat *et al.*, 1990) and the suppression of T lymphocytes *in vivo* requires the presence of spermine oxidase or related enzymes (Quan *et al.*, 1990). Physicochemical characterization of human seminal plasma components provides evidence that transglutaminase and prostaglandins are the principal molecules contributing to seminal plasma immunosuppression of natural killer cells (Tarter *et al.*, 1986; Ablin *et al.*, 1990). Quayle *et al.* (1989) demonstrated that prostaglandins of the E series are almost entirely responsible for the immunosuppression of the natural killer cell system. However, the immunosuppressive component in boar seminal plasma has

no effect on the activity of natural killer cells (Bouvet *et al.*, 1987; Veselský *et al.*, 1992).

Human seminal plasma is lymphocytotoxic at high concentrations (Allen and Roberts, 1986) and the toxic effect of seminal plasma is enhanced by the presence of fetal calf serum. This serum contains a polyamide oxidase that oxidases spermine and spermidine to produce aldehydes that are highly cytotoxic. However, Szymaniec *et al.* (1987) showed that human seminal plasma suppresses lymphocyte responses *in vitro* in serum-free medium. We isolated and characterized a protein from boar vesicle gland secretion that inhibits the proliferation of murine lymphocytes *in vitro* and *in vivo* (Veselský *et al.*, 1991). Immunosuppressive activity of the immunosuppressive component *in vivo* excluded the possibility of artefacts due to interactions with culture media.

In the present report male and female BALB/c mice were used to investigate the effect on cellular immunity of an immunosuppressive fraction isolated from boar vesicular gland secretion and administered to experimental animals by rectal infusion.

Materials and Methods

Isolation of immunosuppressive fraction

The immunosuppressive fraction was isolated from boar vesicular gland secretion using the procedure described by Veselský *et al.* (1991). Vesicular gland secretion was precipitated in 8% ethanol at pH 7.2 and -2.5°C . Samples, 100 mg, of the precipitate were dialysed and dissolved in PBS at pH 7.2, and then applied to a Sephacryl S-200 column (2.4 cm $\times$ 63 cm) equilibrated with PBS, pH 7.2, at a flow rate of 4.2 ml for 15 min. The fractions with inhibitory activity on leucocytes were pooled and run through a Sephadex G-75 column (1.4 cm $\times$ 94 cm), equilibrated with PBS, pH 7.2, at a flow rate of 1.5 ml for 20 min.

Animals and treatments

The effect of rectally infused immunosuppressive fraction on mouse lymphocytes was evaluated in two experiments. In the first experiment, five male and five female BALB/c inbred mice were anaesthetized with ether, and 0.5, 1 or 2 mg of immunosuppressive fraction in 0.1 ml of saline was deposited in the rectum, by means of a catheter to a depth of 4 cm, on days 0, 3 and 6. This treatment did not damage any part of rectal mucosa as faeces passed afterwards were examined and found to be heme negative. Control mice were treated with saline or BSA in the same manner. In all mice, spleen lymphocytes were separated 3 days after the last deposition and assayed in the mitogen-induced lymphocyte proliferation test.

In the second experiment the effect of immunosuppressive fraction *in vivo* was evaluated by the plaque-forming assay. Five male and five female mice were rectally infused with 0.5, 1 or 2 mg of immunosuppressive fraction in 0.1 ml of saline and at the same time injected i.p. with 1×10^8 sheep erythrocytes. Sheep erythrocytes were obtained from blood collected into heparinized tubes by venepuncture of donor animals. After 24 and 48 h, the mice received another rectal infusion of 0.5, 1 or 2 mg of immunosuppressive fraction. Control mice were treated with saline or BSA (2 mg per mouse). Spleen lymphocytes were separated on day 5 after the first rectal infusion and tested in the plaque-forming assay.

Mitogen-induced lymphocyte proliferation test

Seventy-two hours after the final rectal infusion of immunosuppressive fraction, the mice were killed by cervical dislocation. The spleens of the treated mice were aseptically removed, minced with fine scissors, suspended in RPMI 1640 medium (Institute for Sera and Vaccines, Prague), and the cell suspension was passed through a fine screen. Splenocytes were washed twice by centrifugation (10 min at 1000 g). The final splenocyte concentration was adjusted to 2×10^6 cells ml^{-1} in RPMI 1640 medium supplemented with 10% BSA, 2 mmol L-glutamine l^{-1} , 100 iu penicillin ml^{-1} and 0.1 mg streptomycin ml^{-1} . The splenocyte cultures, performed in triplicate in 96-well flat-bottomed culture plates, were stimulated with 0.005 mg pokeweed mitogen (Sigma, St Louis, MO) or 0.05 mg phytohaemagglutinin (Wellcome Research Laboratories, Dartford) ml^{-1} RPMI 1640 medium. The lymphocytes were cultured for 72 h at 38°C. After 48 h of culture, 37 kBq methyl-[^3H]thymidine (specific activity 840 GBq mmol^{-1} , Institute for Research, Production and Application of Radioisotopes, Prague) per well was added. The cells were collected onto glass-fibre discs using a semi-automatic harvester and the radioactivity was measured using a standard liquid scintillation technique. In all cultures, the viability of the lymphocytes was greater than 95% as determined by trypan blue exclusion.

Plaque-forming assay

Experimental animals were killed by cervical dislocation and spleen lymphocytes were collected on day 5 after immunization with sheep erythrocytes. Splenocytes, 0.1 ml (10^6 cells ml^{-1} RPMI 1640 medium), were mixed with 0.05 ml 10% sheep erythrocytes and 0.05 ml guinea-pig complement (Insti-

tute for Sera and Vaccines, Prague) preabsorbed with sheep erythrocytes. The mixture was pipetted into a chamber made of two microscope slides. After incubation for 2 h at 37°C, the plaque-forming cells were counted.

Statistical analysis

Statistical significance of the results was calculated using Student's *t* test.

Results

In the five male and five female mice treated with 1 or 2 mg of immunosuppressive fraction, the blastogenic response of lymphocytes stimulated by pokeweed mitogen and phytohaemagglutinin was significantly reduced ($P < 0.01$) (Table 1). The experiments were repeated three times with the same number of mice per group. The inhibition of the blastogenic response by the immunosuppressive fraction was comparable among experimental groups. No sex-related difference in sensitivity to the immunosuppressor was observed.

A similar inhibitory activity was obtained in the experiments *in vivo*. The rectal infusion of 2 mg immunosuppressive fraction caused 68% ($P < 0.01$) suppression of plaque-forming cells. With 1 mg immunosuppressive fraction the inhibition was 52% ($P < 0.01$). With the infusion of 0.5 mg, the suppression was only 10% (Table 2). The experiments were repeated three times with five male and five female mice per group. The suppression of plaque-forming cells by immunosuppressive fraction was comparable among experimental groups and no sex-related difference was observed.

Discussion

Seminal plasma suppresses a variety of immunological functions *in vitro* and *in vivo* (Quayle *et al.*, 1989; Veselský *et al.*, 1991) and recent studies have indicated that rectal insemination of homologous semen into rabbits had several consequences. Spermatozoa in the rectum evoked the formation of antibodies (Richards *et al.*, 1984), in spite of the fact that seminal plasma is immunosuppressive (Kelly, 1991). It was concluded in studies of natural killer cell function that prostaglandins in semen are responsible for this inhibition (Quayle *et al.*, 1989). It was also demonstrated that cellular immune functions, including natural killer cell activity and mitogen stimulation, were not reduced after a single rectal insemination of human seminal plasma into Rhesus monkeys. Prostaglandin E_2 metabolite values in blood plasma were high in the inseminated monkeys but not in the control monkeys (Alexander *et al.*, 1986). Intra-rectal infusion of PGE_2 or PGD_2 into male rats reduced responses of T cells to phytohaemagglutinin *in vitro* but the T-cell response of female rats was not significantly reduced (Kuno *et al.*, 1986). Boar seminal plasma contains extremely low concentrations of prostaglandins (0.01 mg ml^{-1}) compared with prostaglandin concentrations in human seminal plasma (0.578 mg ml^{-1}) (Mann and Lutwak-Mann, 1981). Moreover, it has been found that immunosuppressive molecules in boar seminal plasma have no effect on cells involved in transplantation events, including

Table 1. Effect of intra-rectal administration of boar seminal immunosuppressive fraction to mice evaluated by mitogen-induced lymphocyte proliferation test

Infusion	PHA (50 µg ml ⁻¹) c.p.m. ± SD	Percentage inhibition	PWM (5 µg ml ⁻¹) c.p.m. ± SD	Percentage inhibition
Saline	70 062 ± 3474		39 658 ± 6174	
Albumin	72 136 ± 4070		37 630 ± 4051	4
ISF (2 mg)	17 071 ± 2859	74	7255 ± 1399	82*
Saline	82 200 ± 6265		21 458 ± 2305	
Albumin	79 672 ± 4563	4	22 931 ± 2751	
ISF (1 mg)	31 352 ± 2759	62*	9428 ± 1739	56*
Saline	29 147 ± 2843		44 426 ± 3858	
Albumin	31 615 ± 3518		46 358 ± 2318	
ISF (0.5 mg)	26 025 ± 2760	11	35 751 ± 3975	19

**P* < 0.01.

ISF: immunosuppressive fraction; PHA: phytohaemagglutinin; PWM: pokeweed mitogen.

Table 2. Effect of intra-rectal administration of boar seminal immunosuppressor to mice evaluated by inhibition of plaque-forming cells

Infusion	Number of mice	Plaque-forming cells (average of group) (± SD)	Percentage inhibition
Saline	5	615 ± 48	
Albumin	5	621 ± 65	
ISF (2 mg)	5	197 ± 29	68*
ISF (1 mg)	5	295 ± 31	52*
ISF (0.5 mg)	5	553 ± 57	10

Values represent the means ± SD for five mice per group. The number of plaque-forming cells was calculated per 10⁶ spleen cells. No sex-related difference in sensitivity to immunosuppressor was observed.

**P* < 0.01.

ISF: immunosuppressive fraction.

natural killer cell activity (Veselský *et al.*, 1992). Our results show that components other than prostaglandins, administered via the rectum cause a decrease in the lymphocyte response *in vivo*. Rectal intercourse is a common sexual activity among homosexual men and, as indicated, the seminal molecules can be absorbed by the recipients via the rectum. A sex-related difference in the sensitivity to prostaglandins has been observed and a possible explanation is that females should be naturally more resistant to prostaglandins (Kuno *et al.*, 1986). Nevertheless, our results indicate that seminal plasma contains other components with immune dysregulation consequences in males and females.

Inhibition of the function of immunocompetent cells would contribute to the development of malignancies and pathological changes caused by infections. Two of the most disturbing diseases associated with sexually transmitted viruses are cervical cancer and acquired immune deficiency syndrome. Sexually transmitted viruses have been proposed as possible carcinogens and Mitchell *et al.* (1986) suggested that the human papilloma virus is a viral agent. The primary agent causing AIDS, the

human immunodeficiency virus (HIV), is found in blood and semen. The most effective way of transmitting HIV is by cell contact. However, the infection was evidenced after the inoculation of HIV into the vaginal mucosa (Fultz *et al.*, 1986). Mucosal transmission of HIV by the vaginal mucosa in heterosexual, or rectal mucosa in homosexual subjects could be responsible for 70–80% of AIDS (Lehner *et al.*, 1991).

Spermatozoa express a number of sperm-specific antigens that can elicit the immune response in sexual partners (Alexander and Anderson, 1987). Although lymphocyte function may be impaired by immunosuppressive factors in seminal plasma, macrophages in semen retain the phagocytic, adherent and motile functions (Anderson and Hill, 1989). Seminal immunosuppressive factors may locally suppress immune defence mechanisms in the female reproductive tract and, thereby, protect spermatozoa and other seminal cells in the female reproductive tract. Potent anticomplement immunosuppressive factors could suppress even humoral defence mechanisms in reproductive tissues (Tarter and Alexander, 1984). In addition, excessive exposure to seminal plasma at sites other than the urogenital tract may have a systemic effect. Seminal components may gain access to the circulation if they are deposited in the gastrointestinal tract, especially if it has been traumatized (James and Hargreave, 1984).

We postulate that absorption of seminal plasma may be a factor in the aetiology of AIDS and other viral infections in males and females. High concentration of immunosuppressive molecules might reduce host defence mechanisms, especially after repeated exposure.

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