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Immunosuppression of Cell- and Serum-Mediated Tumour-Associated Immunity in Prostatic Cancer by Human Seminal Plasma

RICHARD J. ABLIN,* RASHID A. BHATTI, IRVING M. BUSH and PATRICK D. GUINAN

*Divisions of *Immunology and Urology, Cook County Hospital and the Hektoen Institute for Medical Research, Chicago, IL 60612, U.S.A.*

Abstract—*The immunosuppressive properties of the hormonal and/or secretory milieu or tumour-elaborated factors (in the case of carcinoma) of the prostate have been hypothesized as contributory to the natural history of prostatic cancer. The effect of normal human seminal plasma (HuSPl) on immunity to tumour-associated antigens and the 'arming' of normal peripheral blood leukocytes (PBL) by cytophilic antibody in the sera of patients with prostatic cancer have been evaluated by leukocyte adherence inhibition, a suggested* in vitro *correlate of cellular immunity. Significant* ($P<0.01$) *suppression of immunity to malignant prostate ranging from* 16 *to* 80% *of the level of reactivity obtained with unincubated patients' PBL was observed in* 22 (88%) *of* 25 *patients following pre-incubation of their PBL with HuSPl. Similarly, pre-incubation of PBL from eight normal adults with HuSPl prior to 'arming' with sera from patients with prostatic cancer resulted in significant suppression of 'arming' by cytophilic antibody ranging from* 10 *to* 60% *of their level reactivity obtained with unincubated and 'armed' PBL and malignant prostate. Suppression of tumour-associated immunity by HuSPl provides further evidence to studies by others demonstrating SPl suppression of a range of* in vitro *immune responses in normal bovine, murine and human hosts. In addition to the possible biological implications of the immunosuppressive properties of SPl, e.g., as directed toward preservation of the species, whereby under normal conditions tolerance to spermatozoa in the male tract and in the female tract, following coitus, is maintained, it is hypothesized on the basis of collation of studies demonstrating experimental prostatic cancer from sensitization by spermatozoa and the relationship of prostatic cancer to repression of sexual activity, that SPl may play a significant role in the natural history of prostatic cancer.*

INTRODUCTION

ADENOCARCINOMA of the prostate is an insidious disease, the natural history of which is characterized by a high incidence of occult foci found on routine autopsy and wide variation in the age of onset of clinical disease [1, 2]. Immunologic studies of patients with prostatic cancer, as recently reviewed [3] have provided evidence of host responses to tumour. However, the magnitude and consistency of these responses appear less than that observed in patients with other solid tumours. As possible explanations for these enigmas, the existence of the prostate as an immunologically privileged site due to its lymphatic anatomy, i.e., afferent lymphatics [4], or immunosuppressive properties of its hormonal and /or secretory milieu or, tumour-elaborated factors (in the case of carcinoma) [3], has been hypothesized.

In an initial investigation of the role of components of the normal secretory milieu of the prostate as contributory to the possible privileged (partial or complete†) status of the prostate, the effect of normal human seminal plasma (HuSPl) on cell-mediated tumour-associated immunity (TAI) [5–9] and 'arming' factor (cytophilic antibody) [10] in patients with prostatic cancer have been evaluated in the present study.

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†'Partial' is used with respect to the 'complete' immunologically privileged status suggested for the brain and testis.

MATERIALS AND METHODS

Preparation of tissue extracts

Extracts of pooled allogeneic malignant prostate and bladder tissues were prepared by solubilization in 3M KCl and purified by precipitation in saturated $(NH_4)_2SO_4$ as previously detailed [9].

The protein concentrations of extracts so prepared as determined by the method of Lowry *et al.* [11] were 2.0 mg of protein/ml for prostate and 3.9 mg of protein/ml for bladder.

Preparation of human seminal plasma

Whole human semen was collected from 14 healthy adult males into sterile plastic containers and centrifuged at 15,000 rev/min at 4°C for 60 min to obtain clarified SPl by a modification of the procedure of Jecht and Poon [12]. Specimens were pooled and stored at −70°C.

The protein concentration of this pool of HuSPl as determined by the method of Lowry *et al.* [11] was 2.8 mg of protein/ml.

Preparation of leukocytes

Peripheral blood leukocytes (PBL) were obtained from eight normal adults, ranging in age from 26 to 67 yr, and 25 patients with a confirmed histological diagnosis of adenocarcinoma of the prostate [13], ranging in age from 57 to 78 yr, by Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) centrifugation using a modification [9] of the method of Boyum [14]. Following washing, PBL were resuspended at a concentration of 1×10^7 cells/ml in RPMI 1640 medium (Grand Island Biological Company, Grand Island, New York) containing 100 i.u. penicillin G/ml and 100 μg streptomycin/ml. Cell viability was determined by trypan-blue dye exclusion.

Serum specimens

Serum was obtained from 14 patients, 61 to 74 yr of age, with a confirmed histological diagnosis of: stage A (localized)—7 patients, and stage D (metastatic)—7 patients, adenocarcinoma of the prostate [13].

Evaluation of tumour-associated immunity

TAI was evaluated by a modification [9] of the tube leukocyte adherence inhibition (LAI) method of Grosser and Thomson [15]. Briefly, PBL, 0.1 ml, at a concentration of 1×10^7 cells/ml were cultured independently with 0.1 ml of pooled allogeneic extract of malignant prostate and bladder at a concentration of 200 μg of protein/culture, as sources of specific and non-specific antigens. Each culture was brought to a final volume of 0.5 ml by addition of RPMI 1640 medium and mixed thoroughly. Cultures were then incubated for 2 hr at 37°C in 5% CO_2 in air in a horizontal position. After incubation, cultures were carefully placed in a vertical position, the contents gently agitated and the number of non-adherent cells counted in quadruplicate with a standard Neubauer haemocytometer (C. A. Hausser and Son, Philadelphia, Pennsylvania). The non-adherence index (NAI) (mean ± S.D.) was determined as:

$$\text{NAI} = \frac{\text{Mean percentage non-adherent cells obtained with specific antigen} - \text{Mean percentage non-adherent cells obtained with non-specific antigen}}{\text{Mean percentage non-adherent cells obtained with non-specific antigen}} \times 100$$

Delineation of specific reactivity of prostatic cancer patients' PBL with malignant prostate (specific antigen) was made as indicated above by concomitant reaction of the patients' PBL with malignant bladder as a source of non-specific antigen.

Effect of human seminal plasma on tumour-associated immunity

The effect of HuSPl on TAI was evaluated by incubation of 0.1 ml of each patient's leukocytes (1×10^7 cells/ml) and 0.3 ml of RPMI 1640 medium with 0.1 ml of HuSPl at a concentration of 280 μg/ml, which was determined as the optimal inhibitory dosage from a dose-response curve, at 37°C for 50 min in 5% CO_2 in air. After incubation, cells were washed twice with RPMI 1640 medium, their viability assessed by trypan-blue dye exclusion, and then they were transferred to disposable glass culture tubes. Reactivity of PBL pre-incubated with HuSPl to specific and non-specific antigens was then determined by the LAI test as described above and the NAI determined.

Effect of human seminal plasma on 'arming' factor (cytophilic antibody)

The effect of HuSPl on the 'arming' of normal PBL [10] was evaluated by incubation of unincubated and PBL pre-incubated with 280 μg/ml of pooled normal HuSPl at 37°C for 50 min in 5% CO_2 in air with 1:2 dilutions of serum from each of seven patients with stage A and stage D prostatic cancer. After incubation, cells were washed twice in RPMI 1640 medium. their viability assessed by trypan-blue dye exclusion, and then they were transferred to disposable glass culture tubes. Reactivity of unincubated and 'armed' PBL and HuSPl pre-incubated and 'armed' PBL with malignant prostate was then determined by the LAI test as described above.

RESULTS

The effect of normal HuSPl on TAI in patients with prostatic cancer and on the 'arming' of normal PBL with serum from patients with prostatic cancer has been evaluated by LAI.

Effect of human seminal plasma on tumour-associated immunity

The percentage of non-adherent cells (mean ±S.D.) and NAI obtained with unincubated and HuSPl pre-incubated PBL following their reaction with allogeneic extracts of malignant prostate and bladder, as sources of specific and non-specific antigens, are shown in Table 1. An insufficient number of PBL obtainable from 11 patients prevented concomitant evaluation of HuSPl pre-incubated PBL with bladder.

In agreement with previous studies of the tissue-specificity of TAI in prostatic cancer patients, reactivity of the patients' PBL with malignant prostate differed significantly ($P<0.01$) when compared with that obtained with malignant bladder, i.e. 20.4 vs 5.4%. However, when PBL were pre-incubated with HuSPl prior to their reaction with malignant prostate, significant ($P<0.01$) suppression of reactivity to prostate was observed. That is, there was reduction in the percentage of non-adherent cells obtained with malignant prostate when allogeneic malignant prostatic extract was reacted with HuSPl pre-incubated PBL from 20.4 to 11.7%.

The degree of reduction in TAI was variable in each patient, and ranged from 16 to 80%. Overall 22 (88%) of the 25 patients evaluated showed significant reduction in TAI. TAI in 3 (12%) patients remained essentially unchanged.

A slight but non-significant ($P>0.05$) increase in reactivity to bladder from 5.4 to 5.8% was observed following the pre-incubation of PBL with HuSPl. This apparent absence of suppression of reactivity of prostatic cancer patients' PBL pre-incubated with HuSPl with malignant bladder points to a possible specific inhibitory effect of HuSPl on TAI in prostatic cancer.

The significance of the degree of suppression of specific reactivity to prostate following pre-incubation of PBL with HuSPl is further exemplified, as shown in Table 1, by the difference in NAI ontained with unincubated PBL and that obtained with PBL pre-incubated with HuSPl of 357.4 vs that of 195.6 ($P<0.01$) when malignant bladder was used as the source of non-specific antigen.

The possibility that the observed inhibitory effect of HuSPl on the reactivity of PBL to the extracts was due to a cytotoxic effect of the extracts or HuSPl was excluded by the

Table 1. Effect of human seminal plasma (HuSPl) on tumour-associated immunity in patients with prostatic cancer

Peripheral blood leukocytes	Mean ±S.D. percentage non-adherent cells obtained with extract of malignant: Prostate (specific antigen)	Bladder (non-specific antigen)	NAI*
Unincubated	20.4 ± 13.1(25)†	5.4 ± 4.4(25)	357.4
Pre-incubated with 280 μg/ml HuSPl	11.7 ± 8.1(25)	5.8 ± 3.9(14)	195.6
Significance (P)	<0.01	>0.05	<0.01

*Obtained with malignant prostate as specific antigen and malignant bladder as non-specific antigen.

†Number of patients evaluated.

observation that the viability (as assessed by trypan-blue dye exclusion) of PBL incubated for 50 min in the culture medium without and with either extract or 280 μg/ml HuSPl was essentially identical i.e., 92 vs 89%.

Effect of human seminal plasma on 'arming' factor (cytophilic antibody)

The percentage of non-adherent cells (mean ± S.E.) obtained with unincubated and 'armed' normal PBL, allogeneic extract of malignant prostate and normal PBL preincubated with HuSPl prior to 'arming' is shown in Table 2.

reacted with malignant prostate, no reduction in responsiveness was noted.

DISCUSSION

The present observations have demonstrated a significant suppression of the effect of normal HuSPl on TAI and of the 'arming' of normal PBL with cytophilic antibody in patients with prostatic cancer as evaluated by LAI. As such, these initial observations in patients with prostatic cancer are in agreement with observations by others demonstrating the suppressive activity of SPl on a range

Table 2 Effect of human seminal plasma (HuSPl) in 'arming' of normal peripheral blood leukocytes with serum from patients with localized and metastatic prostatic cancer

	Mean ± S.E. percentage non-adherent cells obtained with extract of malignant prostate and normal leukocytes†		
Serum from stage*:	Unincubated and 'armed'	Pre-incubated with HuSPl and 'armed'	Significance (*P*)‡
A (Localized)	24.1 ± 2.7	12.8 ± 1.7	<0.05
D (Metastatic)	13.3 ± 2.0	7.5 ± 2.0	<0.05
Significance (*P*)‡	<0.05	>0.05	

*Serum from 7 patients with localized prostatic cancer (stage A) and 7 patients with metastatic prostatic cancer (stage D).
†From 8 normal adults.
‡Paired *t*-test.

In keeping with previous studies of 'arming' of normal PBL, the reactivity of unincubated PBL 'armed' with serum from patients with stage A disease and malignant prostate was significantly ($P<0.05$) greater than that obtained when the same PBL were 'armed' with serum from patients with stage D disease and reacted with malignant prostate, i.e., 24.1 vs 13.3%. However, when the same PBL were pre-incubated with HuSPl prior to 'arming', significant ($P<0.05$) suppression of their reactivity with malignant prostate was observed. That is, as shown in Table 2, there was a reduction in the percentage of non-adherent cells obtained when malignant prostate was reacted with HuSPl pre-incubated and 'armed' PBL, i.e., from 24.1 to 12 8% with stage A serum and from 13.3 to 7.5% with stage D serum. And, while a difference between the reactivity of PBL, pre-incubated with HuSPl and 'armed' with serum from stage A and stage D disease and malignant prostate remained, i.e., 12.8 vs 7.5%, this difference was no longer significant ($P>0.05$).

When normal PBL were 'armed' with serum prior to incubation with HuSPl and

of other types of *in vitro* immune responses in normal bovine, murine and human hosts [16–20].

Identification of the specific component(s) of SPl responsible for its suppressive activity require further study. Nonetheless, among other biological implications, suppression by SPl may represent a means of preservation of the species, whereby under normal conditions tolerance to spermatozoa in the male and in the female, following coitus, are maintained.

By the same mechanism, immunosuppression of TAI by SPl may contribute to the failure of the prostatic cancer patient to develop a substantial response to his malignancy in the early stages of disease. For example, studies of serum antibody [21] and of as yet unpublished observations of cellular responsiveness (Bhatti, unpublished observations) have demonstrated greater levels of TAI in patients with metastatic (stage D) disease than in patients with localized (stage A) disease. Furthermore, the availability and binding of cytophilic antibody to monocyte receptors may be critical to 'arming' and possibly in the presentation of antigen to reactive cells requisite for the induction of

various immune responses. As such, immunosuppression of cytophilic antibody by SPl may maintain host unawareness to aberrant cells and contribute to progression of asymptomatic disease.

Therefore, on the basis of the: (i) immunosuppressive properties of SPl demonstrated in this and previous studies [16–20]; (ii) correlation of prostatic cancer with repression of sexual activity [22] and (iii) experimental induction of prostatic cancer from sensitization by spermatozoa penetrating prostatic tissue [23], it is further hypothesized [24] that accumulation of unejaculated spermatozoa, principally under conditions (psychological or physiological) placing limitations upon sexual activity, penetrate and sensitize the prostate under suboptimal conditions, e.g., chronic prostatitis, inducing the neoplastic process which culminates in a silent (occult) asymptomatic carcinoma with no substantial stimulation of host responsiveness, despite aberrant tissue antigens, because of the milieu, i.e., seminal plasma.

Obviously, this hypothesized phenomena is not an all or none situation and reasonably not the sole or, perhaps, even principal mechanism operative in contributing to the etiology of prostatic cancer. It may, however, particularly with regard to the suppressive effect of SPl on TAI, offer some explanation for the high incidence of latent and occult prostatic cancer and the wide variation in the age of onset of clinical disease.

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