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Cell-mediated Immunity in Prostatic Cancer and its Diagnostic Relevancy

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Abstract—*Cell-mediated immunity (CMI) in prostatic cancer patients to presumptively identified prostatic tumour-associated antigens (TAA) was further evaluated in this study by tube leukocyte adherence inhibition in 504 patients with and without prostatic cancer. Peripheral blood leukocytes from 210 of 312 (67%) prostatic cancer patients possessed significant reactivity to extracts of malignant prostate. However, significant reactivity to malignant prostate was also observed in 89 of 192 (46%) controls comprised of patients with other than carcinoma of the prostate [including 91 patients with benign prostatic hypertrophy of which 46 (51%) possessed significant reactivity to malignant prostate] and healthy adults. With the exception of a significant difference in the reactivity between stage A vs stage C patients, there was no significant correlation between the level of reactivity to malignant prostate and the stage of disease. Had CMI to presumptively identified prostatic TAA been employed as an adjunctive diagnostic criterion to detect prostatic cancer, 191 (38%) of the 504 patients in this study would have been incorrectly diagnosed. The results of this study emphasize the critical need in attempting to delineate tumour-directed immunity from possible concomitant sensitization to tissue- and species-specific antigens for the identification, isolation and physicochemical characterization of what previously have been referred to as presumptively or putatively identified prostatic TAA.*

INTRODUCTION

FOR THE most part, cell-mediated immune (CMI) responses to human tumours are weak and inconsistent, and their exact nature and specificity have been difficult to define [1]. Prostatic cancer is no exception [2].

In a recent report [3], we communicated the results of our studies of the evaluation of CMI in patients with prostatic cancer to presumptively identified prostatic tumour-associated antigens (TAA). Employing slide and tube leukocyte adherence inhibition (LAI) as the *in vitro* immunological criteria, the results of that study [3] suggested that further evaluation of the usefulness of identifying significant levels of CMI to prostatic TAA as a possible adjunct to existing methods of diagnosis of prostatic cancer was indicated. As such, we have extended our initial study of CMI in 73 prostatic cancer patients to 312 patients, the results of which are presented herein and compared with those obtained in 192 non-prostatic cancer control patients, including 91 patients with benign prostatic hypertrophy.

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MATERIALS AND METHODS

Patients and controls

Three hundred and twelve patients with adenocarcinoma of the prostate as confirmed by histological diagnosis and staged in accord with conventional protocol [4] were evaluated. These patients ranged in age from 59 to 90 yr and were comprised of 20 patients with stage A, 53 with stage B, 34 with stage C and 205 patients with stage D disease. All patients were receiving conventional treatment at the time of their evaluation in this study.

One hundred and ninety-two individuals, comprised of 91 patients with benign prostatic hypertrophy (BPH), 27 with carcinoma other than of the prostate and 74 healthy adults, as detailed in Table 2, served as controls.

Table 1. Evaluation of the tissue-specificity of the reactivity of leukocytes from patients with prostatic cancer to malignant prostate

Allogeneic extract of:	Mean ± S.E. percentage of non-adherent cells	NAI*
Normal prostate (102)†	10.3 ± 0.9	166.0
Benign prostate (56)	6.5 ± 1.0	322.0
Malignant prostate (312)	27.4 ± 3.3‡	—

The NAI indicated is that for the reactivity of prostatic cancer patients' leukocytes determined from using malignant prostate as specific extract, with extracts of normal and/or benign prostate as non-specific extract(s).

$$*\text{NAI} = \frac{\text{mean \% non-adherent cells obtained with specific extract} - \text{mean \% non-adherent cells obtained with non-specific extract}}{\text{mean \% non-adherent cells obtained with non-specific extract}} \times 100.$$

†No. of patients evaluated with extract indicated.

‡Significance (P) between the mean percentage of non-adherent cells obtained with leukocytes from patients with prostatic cancer and malignant prostate vs that obtained with normal and benign prostate $P < 0.05$.

Tissue extracts

Extracts in 3M KCl $(NH_4)_2SO_4$ were prepared from allogeneic normal, benign and malignant* prostatic tissues as detailed previously [3].

Leuokocytes

Peripheral blood leukocytes (PBL) were isolated from the leukocyte-rich plasma of heparinized blood obtained from patients and controls by centrifugation on a Ficoll-Isopaque gradient and prepared for use in the LAI test as described previously [3].

Leukocyte adherence inhibition test

The tube LAI test was carried out as previously detailed [3]. In brief, 3M KCl $(NH_4)_2SO_4$ extracts of malignant prostate (specific extract) were reacted at a protein concentration of 400 μg/culture with patient and control PBLs. Pending their availability, patient and control PBLs were concomitantly and independently reacted with extracts of normal and/or benign prostate as sources of non-specific extracts.

The results are expressed as the mean ± S.E. per cent of non-adherent cells obtained with each of the extracts as counted in quadruplicate by the non-adherence index (NAI), and the significance of the difference in the reactivities obtained between the extracts and patient and control PBLs.

RESULTS

In this study the degree of tissue- and disease-specificity of the reactivity of malignant prostate with leukocytes from 312 patients with prostatic cancer and the relationship of this reactivity to their stage of disease was evaluated in comparison to that in 192 patients with other than prostatic cancer (controls) by tube LAI.

Tissue-specificity

The tissue-specificity of the reactivity of prostatic cancer patients' leukocytes to malignant prostate was evaluated by comparison of their degree of reactivity with allogeneic extracts of normal and benign prostate.

As shown in Table 1, varying degrees of specificity, expressed by the mean percentage of non-adherent cells and NAI, were observed. This variability was dependent on the source of non-specific extract, e.g. normal prostate, used to determine the NAI.

Absence of significantly different ($P > 0.05$) reactivity to that obtained with malignant prostate with normal and benign prostatic tissues, i.e. 27.4 vs 10.3 and 6.5% respectively, suggests that the observed reactivity of prostatic cancer patients leukocytes is directed toward prostate TAA.

Disease-specificity

To evaluate the disease-specificity of the reactivity of prostatic cancer patients' leukocytes with malignant prostate, leukocytes from 192 patients with other than prostatic cancer (controls) were reacted with malignant prostate.

As shown in Table 2, the mean reactivity of 27.4% obtained for the prostatic cancer patients following reaction of their leukocytes with malignant prostate differed significantly ($P < 0.05$) from the mean of 14.9% obtained for the reactivity

*A portion of the pool of the malignant prostatic tissues was obtained from the National Prostatic Cancer Project, University of Miami, Miami, FL.

of leukocytes from the controls reacted with malignant prostate.

Based on the mean percentage of non-adherent cells of 14.9% obtained with the controls, the mean percentage of non-adherent cells of ⩾19.7%, i.e. the mean of 14.9 + 2 S.E., was considered to indicate significant reactivity to malignant prostate. On this basis, 210 (67%) of the 312 prostatic cancer patients evaluated possessed significant reactivity to malignant prostate. Of the controls, 89 (46%) possessed ⩾19.7%. The number of patients in each of the three groups of controls, i.e. those with BPH, carcinoma other than of the prostate and healthy adults, possessing significant levels of reactivity to malignant prostate is further indicated in Table 2.

Relationship with stage of disease

When the prostatic cancer patients were categorized in accord with their stage of disease, variable degrees of significantly different ($P < 0.01$ to $P < 0.05$) reactivity were noted, as shown in Table 3, in each stage compared to that obtained in the controls. However, with the exception of a significant ($P < 0.05$) difference between the mean reactivity in stage A (31.0%) vs stage C (23.1%), differences between the other stages were not significant.

While the mean levels of reactivity for each of the stages differed significantly from the controls, there was, as shown in Table 3, a variable significant decrease in the number of prostatic cancer patients with significant levels of reactivity to malignant prostate, i.e. ⩾19.7%, in those with stage B disease or greater. Particularly striking in this regard was the 90% (18 of 20) positivity of significant reactivity to malignant prostate in patients with stage A disease.

DISCUSSION

Extension of our initial observations of CMI to presumptively identified prostatic TAA in patients with prostatic cancer [3] and assessment of its relevancy as a potential adjunct to existing methods of diagnosis for prostatic cancer has been the subject of this study.

Table 2. Evaluation of the disease-specificity of the reactivity of leukocytes from patients with prostatic cancer to malignant prostate

Diagnosis	No. of patients	Mean ± S.E. percentage of non-adherent cells obtained with allogeneic extract of malignant prostate	No. of patients (%) with significant reactivity to malignant prostate
Carcinoma of the prostate	312	27.4 ± 3.3†	210 (67%)
Benign prostatic hypertrophy	91	17.6 ± 1.2	46 (51%)
Carcinoma other than of the prostate‡	27	13.7 ± 3.6	7 (26%)
Healthy adults§	74	15.9 ± 1.2	36 (49%)
All patients other than carcinoma of the prostate	192	14.9 ± 2.4	89 (46%)

*Based on the mean percentage of non-adherent cells of 14.9% obtained with the 192 'all patients other than carcinoma of the prostate' control troup, a mean percentage of non-adherent cells ⩾19.7%, i.e. the mean +2 S.E., was considered to indicate significant reactivity to malignant prostate.

†Significance (P) exists between the mean percentage of non-adherent cells obtained with leukocytes from patients with carcinoma of the prostate and malignant prostate vs that obtained with each of the control groups, e.g. patients with benign prostatic hypertrophy, and all patients other than carcinoma of the prostate ($P < 0.05$).

‡Included are 17 patients with carcinoma of the bladder, 6 of the penis and 4 of the testis.

§Included are laboratory personnel, hospital staff and patients admitted for circumcision and renal calculi, with no evidence of diseases of the prostate or of malignancy.

Table 3. Relationship of reactivity of leukocytes from patients with prostatic cancer to malignant prostate according to their stage of disease

Stage	No. of patients	No. of Mean ± S.E. percentage of non-adherent cells patient obtained with allogeneic extract of malignant prostate*	No. of patients(%) with significant reactivity to malignant prostate†
A	20	31.0 ± 3.8	18 (90%)
B	53	28.7 ± 2.1	37 (70%)
C	34	23.1 ± 1.9	21 (62%)
D	205	26.9 ± 1.1	134 (65%)
Controls	192	14.9 ± 2.4	89 (46%)

*Significance (P) between prostatic cancer patients and controls $P < 0.01$ to < 0.05, between Stage A vs Stage C $P < 0.05$, between other stages $P > 0.05$.

†⩾19.7% non-adherent cells as defined in Table 2.

As evaluated by tube LAI, the degree of tissue-specificity of the reactivity of prostatic cancer patients' leukocytes with malignant prostate was in consonance with the results of our previous study [3].

However, concomitant with an increase in the number of patients and controls evaluated, a decrease in the number of patients with significant reactivity to malignant prostate from 77 (56 of 73) to 67% (210 to 312) and an increase in the number of controls with significant reactivity to malignant prostate from 0 (0 of 52) to 46% (84 of 192) were observed. As such, 102 (33%) of the 312 prostatic cancer patients were evaluated as false negatives and 89 (30%) of the 192 controls were evaluated as false positives. On this basis, had the CMI to presumptively identified prostatic TAA been employed as an adjunctive diagnostic criterion to detect which of the 504 patients evaluated in this study had prostatic cancer, 191 patients (38%) would have been incorrectly diagnosed.

With the exception of a significant difference in reactivity between stage A vs stage C patients, differences between other states, as in our initial study [3], were not significant.

The results of this study are, save for their possibly marginal prognostic value once a diagnosis has been established, disappointing as to the potential of employing CMI to presumptively identified prostatic TAA as an adjunct to existing methods of diagnosis for prostatic cancer.

Comparison of the present results with the recent report by Kaneti *et al.* [5], also employing tube LAI to evaluate CMI in prostatic cancer patients, is not possible as they employed extracts of secondary, i.e. metastatic, prostatic tumours rather than of the primary tumour.

The relatively high percentage of the controls having a significant level of reactivity to malignant prostate suggests that this reactivity may have been directed to prostatic tissue-specific and ubiquitous species-specific rather than solely prostatic TAA. Sensitization to prostatic tissue-specific antigens is not that unusual considering prostate antigens are androgenically [6–9] and ontogenically [10] dependent, with some arising for only the first time in puberty. With such a late onset in development, tolerance, i.e. recognition by the immune system of 'self' from 'non-self', would not have developed to these normal antigens. Thus the reaction of the immune system to prostatic tissue-specific antigens would be much the same as that to a foreign antigen.

The outcome of this and previous dilemmas in attempting to delineate tumour-directed immunity in patients with prostatic cancer from possible concomitant sensitization to prostatic tissue- and species-specific antigens is the obvious need for the identification, isolation and physicochemical characterization of what previously have been referred to as presumptively [3, 11, 12] or putatively [13] identified prostatic TAA, and the re-evaluation of patients and controls with such characterized antigens. Until this is done, results of studies such as the present will continue to have their obvious limitations.

REFERENCES

1. REIF AE. *Immunity and Cancer in Man.* New York, Marcel Dekker, 1975.
2. ABLIN RJ, BHATTI RA. Tumor-associated immunity in prostatic cancer. In: ABLIN RJ, ed. *Prostatic Cancer.* New York, Marcel Dekker, 1981, 183–204.
3. ABLIN RJ, BHATTI RA, NAGUBADI S, ZAMORA S, GUINAN P. Cell-mediated immunity in prostatic cancer. *Clin Oncol* 1980, **6**, 253–264.
4. SCOTT WW, SCHIRMER HKA. Carcinoma of the prostate. In: CAMPBELL WW, HARRISON HW, eds. *Urology.* Philadelphia, W. B. Saunders, 1970, 1143.
5. KANETI J, THOMSON DMP, REID EC. Prostaglandin E_2 affects the tumor immune response in prostatic cancer. *J Urol* 1981, **126**, 65–70.
6. STOLL HW, ANSELL JS, BARNES MS, BARNES GW. The autoimmune response to male reproductive tissues of rabbits. III. Effects of testosterone and estrogen on cryoimmunogenic and immunochemical expressions of the central accessory glands. *Invest Urol* 1974, **12**, 236–245.
7. ABLIN RJ, BARNES GW, STOLL HW. Cryostimulation. Androgenic and ontogenic dependence of the immune response following *in situ* autosensitization. (Abstract.) *Cryobiology* 1975, **12**, 571.
8. VENEZIALE CM, BURNS JM, LEWIS JC, BUCHI KA. Specific protein synthesis in isolated epithelium of guinea-pig seminal vesicle. Effects of castration and androgen replacement. *Biochem J* 1977, **166**, 167–173.
9. PARKER MG, SCRACE GT, MAINWARING WIP. Testosterone regulates the synthesis of major proteins in rat ventral prostate. *Biochem J* 1978, **170**, 115–121.
10. ABLIN RJ. Cryosurgery of the rabbit prostate. Comparison of the immune response of immature and mature bucks. *Cryobiology* 1974, **11**, 416–422.

11. ABLIN RJ, MARROW C, GUINAN PD, BRUNS GR, SADOUGHI JT, BUSH IM. Evaluation of cellular immunologic responsiveness in the clinical management of patients with prostatic cancer. III. Inhibition of leukocyte migration. *Urol Int* 1976, **31**, 444–458.
12. EVANS CM, BOWEN JG. Immunological tests in carcinoma of the prostate. *Proc R Soc Med* 1977, **70**, 417–420.
13. WRIGHT GL JR, SCHELLHAMMER PF, FAULCONER RJ, REID JW, BRASSIL DN, SIEG SM. Leukocyte migration inhibition assay in patients with prostate adenocarcinoma. *Prostate* 1981, **2**, 121–131.