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Leukocyte Adherence Inhibition and Immunoreactivity in Prostatic Cancer. II. Tissue- and Disease-Specificity of Anti-Tumour Cell-Mediated Immunity

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Abstract—*Tissue- and disease-specificity of the reactivity of peripheral blood leukocytes from 20 prostatic cancer patients to malignant prostatic tissue have been evaluated. Results of leukocyte adherence inhibition, employed previously, had suggested antitumour cell-mediated immunity. Absence of significant reactivity in prostatic cancer patients to tissues other than malignant prostate and in non-prostatic cancer patients (including patients with benign disease of the prostate) to malignant prostate confirms earlier observations. These had suggested the identification of prostatic tumour-associated antigens and of tumour-associated immunity in prostatic carcinoma.*

INTRODUCTION

STUDIES directed toward assessment of the immunological responsiveness of patients with prostatic cancer and possible modification of this response to augment tumour-host immunity have recently been considered and reviewed [1, 2].

Initial results obtained utilizing leukocyte adherence inhibition (LAI) [3] to assess immunological responsiveness to tumour in prostatic cancer suggest that this may be a reproducible and technically feasible assay. Pending delineation of the tissue- and disease-specificity of the initially suggested anti-tumour associated immunity (TAI) to prostatic tumour-associated antigens (TAA) in patients with prostatic cancer, LAI may provide an immunological means to augment present methods of diagnosis of prostatic cancer.

To this aim, the results of our initial studies of the tissue- and disease-specificity of the responsiveness of leukocytes from 20 patients with prostatic cancer to malignant prostate, as evaluated by LAI, are herein reported.

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MATERIALS AND METHODS

Patients

Twenty patients with a confirmed histological diagnosis of adenocarcinoma of the prostate receiving conventional treatment, initially evaluated for the reactivity of their leukocytes to extracts of malignant prostate [3], were further studied to ascertain the tissue- and disease-specificity of this reactivity. Patients ranged in age from 62 to 75 yr and, as staged in accord with conventional protocol [4], were comprised of 3 patients with stage A, 2 with stage B, 2 with stage C and 13 patients with stage D.

Controls

To ascertain the disease-specificity of reactivity with malignant prostate, 13 age-related patients with other than carcinoma of the prostate, including 3 patients with prostatitis, were evaluated. All diagnoses are indicated in Table 2.

Tissue extracts

Extracts of allogeneic malignant prostatic

tissue obtained at the time of transurethral resection and necropsy were prepared as initially described [3]. For this purpose, 2 pools of 3M KCl extracts of prostatic tissue from 9 and 12 patients, respectively, with histologically confirmed adenocarcinoma of the prostate were prepared. The protein concentrations of these two pools, determined by the Lowry method [5], were 1.0 and 3.8 mg/ml, respectively. As these extracts had possessed similar degrees of reactivity in a pilot study of 8 prostatic cancer patients [6], they were pooled. This pool was used in the previous [3] and present evaluation of anti-tumour cell-mediated immunity which spanned a period of approximately 5 months. During this time no appreciable loss in extract activity was noted.

Extracts of normal prostate (from 3 patients) and malignant bladder and kidney (renal cell carcinoma) (from 4 and 3 patients, respectively) were prepared identically [3] and possessed protein concentrations ranging from 2.0 to 3.8 mg/ml.

Cells

Peripheral blood leukocytes (PBL) were obtained, as described [3], from the leukocyte-rich plasma of heparinized blood on a Ficoll–Isopaque gradient. Cells were washed in minimum essential medium and resuspended in RPMI 1640 medium containing 100 U/ml penicillin G and 100 μg/ml streptomycin at a concentration of 2×10^7 cells/ml. Cell viability was determined by trypan-blue dye exclusion.

Leukocyte adherence inhibition test

Modified after the initial method of Halliday and Miller [7], LAI was carried out as previously described [3].

In brief, 0.1 ml of PBL at a concentration of 2×10^7 cells/ml were cultured independently with equal volumes of: (i) homologous (normal human serum (NHS)) and (ii) 3M KCl extracts of pooled allogeneic normal or malignant tissue +NHS at 37°C for 30 min. LAI was then determined by introducing each culture into the two chambers of Standard Neubauer haemocytometers, incubation at 37°C for 60 min. in a humidified atmosphere of 5% CO_2 in air mixture, and counting the number of adhering nucleated cells prior to and following washing.

RESULTS

The tissue- and disease-specificity of the reactivity of leukocytes from 20 patients with prostatic cancer with malignant prostatic tissue has been evaluated.

Evaluation of tissue-specificity

The results obtained in evaluating the reactivity of prostatic cancer patients' leukocytes with allogeneic extracts of normal prostate and non-prostatic malignant tissues (bladder and kidney) are compared to those obtained with malignant prostate (Table 1). As not all 20 patients were evaluated with each of the extracts, except for malignant prostate, the number of patients evaluated with each extract is indicated.

As shown in Table 1, significant ($P<0.05$) differences were observed between the reactivity (expressed as the mean per cent adherence) obtained between prostatic cancer patients' leukocytes with malignant prostate (22%) and that obtained with normal prostate (52%), or malignant bladder (63%) or kidney (60%).

These observations are suggestive of a high degree of tissue-specificity of the reactivity of

Table 1. Reactivity of prostatic cancer patients' leukocytes with prostatic and non-prostatic tissue extracts as evaluated by leukocyte adherence inhibition

Extract	Mean ± S.D. per cent adherence:	Significance (P)†
Prostate:		
Normal (8)*	52 ± 16	<0.05
Malignant (20)	22 ± 8	—
Malignant:		
Bladder (8)	63 ± 6	<0.05
Kidney (12)	60 ± 17	<0.05

*Number of patients evaluated with extract indicated.
†P value indicated is that obtained in comparing the reactivity of prostatic cancer patients' leukocytes with malignant prostate vs that obtained with normal prostate, malignant bladder or kidney.

Table 2. Reactivity of leukocytes from patients with and without prostatic cancer to allogeneic extracts of malignant prostatic tissue as evaluated by leukocyte adherence inhibition

Diagnosis	Number of patients	Mean ± S.D. per cent adherence	Significance (P)‡
Carcinoma of the prostate	20	22 ± 8	—
Prostatitis	3	89 ± 7	<0.05
Carcinoma other than of the prostate*	4	65 ± 7	<0.05
Non-malignant diseases†	2	55 ± 13	<0.05
Healthy adults	4	64 ± 14	<0.05
All patients other than carcinoma of the prostate	13	69 ± 15	<0.05

*Included 3 patients with renal cell carcinoma and one with mammary carcinoma.
†Two patients with renal cyst.
‡P value indicated is that obtained in comparing the reactivity of prostatic cancer patients' leukocytes with malignant prostate vs that obtained with leukocytes from patients with other than prostatic cancer and malignant prostate.

prostatic cancer patients' leukocytes for malignant prostate. This reactivity appears to be directed toward prostatic TAA.

Evaluation of disease-specificity

In evaluating the disease-specificity of the reactivity of prostatic cancer patients' leukocytes to malignant prostate, significant differences were noted between the mean per cent adherence obtained for prostatic cancer patients (22%) vs that obtained between non-prostatic cancer patients' leukocytes and malignant prostate (Table 2).

Based on the mean per cent adherence of 69% obtained with non-prostatic cancer patients, a mean per cent adherence <39% (i.e., the mean of 69%-2S.D.) was considered to indicate significant reactivity to malignant prostate. On this basis, 16 (80%) of the 20 patients with prostatic cancer possessed significant reactivity. None of the 13 non-prostatic cancer patients, including 3 patients with prostatitis, possessed significant reactivity to malignant prostate.

DISCUSSION

In the present study, LAI has been employed to evaluate the tissue- and disease-specificity of the reactivity of leukocytes from patients with prostatic cancer. It had previously been suggested [3], on the basis of their reactivity with extracts of malignant prostate, that these leukocytes possessed TAI to prostatic TAA.

Of 20 prostatic cancer patients evaluated, 16 (80%) of whom previously demonstrated significant reactivity to malignant prostatic tissue [3], none possessed significant reactivity to extracts of normal prostate or of non-prostatic malignant tissues (bladder and kidney). These observations are suggestive of a high degree of tissue-specificity of the reactivity of prostatic cancer patients' leukocytes for malignant prostate. This reactivity appears to be directed to what have been presumptively identified, pending their isolation and physicochemical characterization, as prostatic TAA.

Similarly, leukocytes from 13 age-related patients with other than carcinoma of the prostate, including 3 patients with prostatitis, did not possess significant reactivity to extracts of malignant prostate. These observations suggest that leukocytes from patients with diseases other than prostatic carcinoma did not possess a significant degree of *in vivo* sensitization (immunity) to prostatic TAA.

The present observations are in consonance with the initially suggested identification of prostatic TAA and of TAI obtained when inhibition of leukocyte migration (ILM) was employed [8]. Contrary to difficulties encountered with the ILM assay [8], LAI appears to provide a reproducible and technically feasible *in vitro* assay of cellular immunologic responsiveness to tumour in prostatic cancer. Implementation of a clinical trial of LAI as a potential routine diagnostic test for prostatic cancer would appear to be particularly attractive in view of the rather high incidence of positive reactivity observed in prostatic cancer patients and of the suggested tissue- and disease-specificity of this reactivity.

Recently completed observations in this laboratory [9] and in that of Bowen and Evans

[10] support implementation of a clinical trial of LAI. When tube LAI was employed, 40 (75%) of 53 (9) and 17 (89%) of 19 (10) prostatic cancer patients, respectively, possessed significant reactivity to malignant prostate.

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