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Oncornavirus-like Protein Expression in Human Prostatic Tissue*

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Summary. A sensitive competition radioimmunoassay using ^{125}I-labelled p 30 interspecies antigen, antiserum specific to the interspecies antigen of the feline leukaemia virus, and aqueous tissue extracts from prostate was used to examine benign hyperplastic prostates for the presence of protein components able to compete with the interspecies viral antigens. Six of 20 prostatic nodular hyperplastic tissues were competitive in radioimmunoassay with the ^{125}I-labelled viral antigen for binding sites on the antiviral antibodies. These findings suggest the presence of oncornavirus-like proteins in prostatic nodular hyperplasia. No correlation could be made between the presence of competing protein and histological features of acute or chronic prostatitis and squamous metaplasia.

Key words: Benign prostatic hypertrophy, Oncornavirus-like proteins, Radioimmunoassay.

The immunology of nodular hyperplastic prostatic tissue has been previously examined. Flocks et al. (5) produced tissue-specific and species-specific rabbit antisera to human prostatic tissue. Specific antigenic differences between benign prostatic hyperplasia and prostatic cancer were not demonstrated. Flocks et al. (4) later demonstrated that pooled homologous dog prostatic protein produced significant antibody titres in dogs with a histological response to the isoantigen demonstrable in dog prostatic tissue. The tissue demonstrated interstitial inflammatory reaction accompanied by interstitial oedema and infiltration of inflammatory cells. This study did not differentiate between antigen derived from carcinomatous prostatic tissue and antigens arising from benign prostatic hyperplastic tissue. In 1970, Ablin and co-workers (2) identified three prostate-specific antigens using double immunodiffusion gel techniques. Two antigens were tissue-specific and the remaining antigen was common to both prostatic tissue and prostatic fluid. Ablin (1) later, using gel diffusion precipitation techniques, detected specific antigenic differences between normal, benign, and malignant prostatic tissue extracts and subsequently postulated that benign and malignant human prostatic tissues are antigenically deficient when compared to normal human prostatic tissue.

The question of a viral aetiology of human carcinoma has been examined extensively during the last several years. Morphological, biochemical and immunological methodology have been used to gather information. The present study was undertaken to determine whether proteins immunologically similar to feline and murine interspecies antigen could be detected in extracts of human prostatic adenocarcinoma. Benign prostatic hyperplasia (B. P. H.) was selected originally as a control tissue. However, when initial studies indicated that the benign prostatic hyperplastic controls might have proteins similar to those being sought in prostatic adenocarcinoma, specific attention was directed to these "control tissues".

MATERIALS AND METHODS

Procurement of Tissues

Nodular hyperplastic prostatic tissue was obtained at surgery under sterile conditions and divided aseptically: one portion was placed in

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tissue culture, one portion was stored in liquid nitrogen for future study, and a third portion extracted for immunological testing.

Tissue Extraction

Tissues were homogenised in a Sorvall Omnimixer in pH 7.6 buffer containing 0.02M Tris-HCl, 0.1 M NaCl, 0.001 M EDTA, and 20 mg/ml crystalline bovine serum albumin (BSA), then treated with 0.4 percent Triton X-100. This was followed by extraction with one volume of ether and centrifugation in a Sorvall RC-2B centrifuge. The aqueous portion of the extract was removed and the ether blown off using gaseous nitrogen. Tissue extracts were standardised to equal protein concentration.

Viral Reagents

Feline (Rickard) and murine (Friend) leukaemia viruses (FeLV, MuLV) were produced in large quantities from continuous suspension culture. The internal proteins from these viruses, containing the interspecies antigen, were then isolated in highly purified form by passage through guanidine gel filtration columns (6). High titre monospecific antisera were raised in rabbits against the interspecies component of both feline and murine virus. The antisera produced were then used in radioimmunoassays.

Labelling

Viral antigens were labelled to high specific activity by a modification of the chloramine T method (7). The polypeptide to be labelled was mixed with 0.025 ml of 0.5 M phosphate buffer, pH 7.5, ^{125}I, and 0.025 ml chloramine T (100 µg). After 2 minutes, sodium metabisulphite, 0.5 ml (120 µg) and potassium iodide, 0.2 ml in 0.005 M phosphate buffer (0.1 mg) were added. Four minutes later the mixture was washed with 0.2 ml potassium iodide (2 mg) and applied to a column (10 mm x 20 cm) packed with Sephadex G-50 which had been pre-washed with 0.05 M phosphate buffer. The fractions containing the greatest concentration of labelled polypeptide were pooled, bovine serum albumin added to a final concentration of 10 mg/ml, and dialysed overnight in a pH 7.6 buffer of 0.02 M Tris-HCl, 0.1 NaCl, 0.001 M EDTA. This resulted in labelling of the viral protein with ^{125}I to specific activity of 10^4-10^5 cpm/ng.

Radioimmunoassay

Monospecific antiserum and ^{125}I-labelled polypeptide antigens were utilised such that the interspecies polypeptide, p30, of both feline and murine leukaemia viruses of known infected cells could be detected in extremely small quantities (10 to 15 nanograms) (11). Five thousand to 10,000 counts per minute of ^{125}I-labelled antigen in 0.02 M Tris-HCl, 0.1 M NaCl, 0.001 M EDTA, (pH 7.6), 20 mg/ml crystalline bovine serum albumin, and 16.5 percent normal rabbit serum were incubated with various serum dilutions at 37°C for 3 hours. A second antibody, goat antirabbit IgG, was added and allowed to complex. After overnight incubation at 4°C, bound and free radiolabelled antigens were separated by centrifugation in an Eppendorf 3200 centrifuge and measured by gamma scintillation. The degree of reaction was indicated by the percentage of radiolabelled antigen in the precipitates. Standard curves of these antigen-antibody reactions were plotted and the appropriate antiserum concentration for competition assays chosen.

Competition Radioimmunoassays

Equal protein concentrations of tissue extracts were allowed to incubate at 37°C with either feline or murine antiserum in 0.02 M Tris-HCl, 0.1 M NaCl, 0.001 M EDTA (pH 7.6), 20 mg/ml crystalline bovine serum albumin, and 16.5 percent normal rabbit serum for 1 hour. Five thousand to 10,000 counts per minute of ^{125}I-labelled antigen were added and incubated at 37°C for 2 hours. Goat anti-rabbit IgG was added followed by overnight incubation at 4°C. Separation and counting were done as described previously. Total reaction volume in individual assay tubes was 90 microliters. Individual assays were run either in duplicate or triplicate and results reconfirmed on subsequent days with repetitive assays. Tissue extracts used in the competition radioimmunoassays were designated antigen positive if they showed a 30% or greater displacement of the labelled antigen used in the assay.

RESULTS

Murine interspecies antigen was labelled with ^{125}I and allowed to react with various dilutions of anti-FeLV p30 serum. The degree of reaction was indicated by the percentage of radiolabelled antigen in the precipitates and allowed the construction of antibody titration curves. The percentage precipitation of 1 ng of ^{125}I-MuLV p30 at various dilutions of anti-FeLV serum is shown in Figure 1. Choosing a dilution of antiserum (1 : 1400) which precipitated approximately 50% of the labelled antigen, and incubating various concentrations of aqueous tumour extracts with this antiserum prior to adding the ^{125}I-MuLV p30, this assay became a competition radioimmunoassay. Figure 2 shows a dose

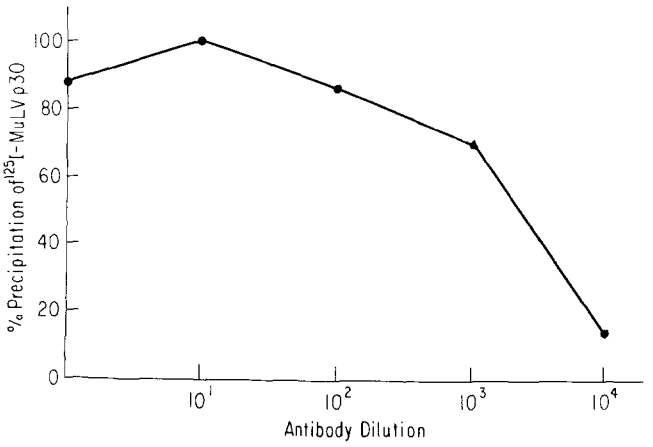

Fig. 1. Antibody titration curve for p30 antigen of type C virus. Antibody is FeLV p30 serum; antigen is 1.0 ng ^{125}I-labelled MuLV p30

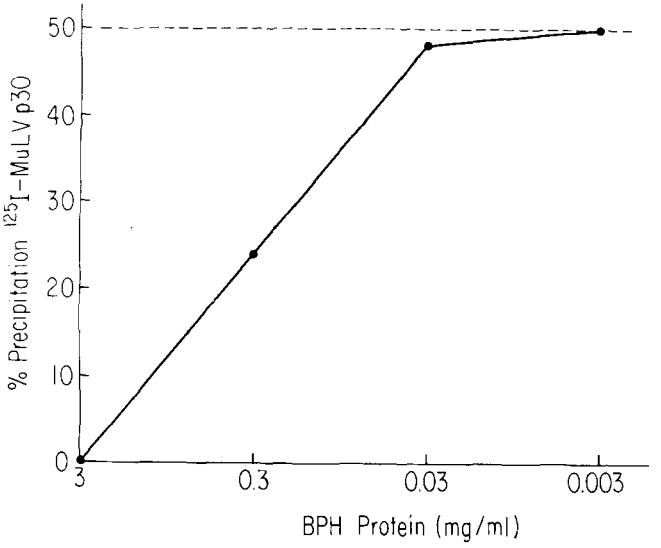

Fig. 2. Competition radioimmunoassay of type C viral protein and extract of BPH tissue. The analysis was performed with 1 : 1400 dilution of FeLV p30 serum, 1.0 ng ^{125}I-labelled MuLV p30 and varying amounts of BPH protein extract

Table 1. Prostatic tissue competitive in radioimmunoassay compared with histological features

Tissue	Mean % competition	Nodular hyperplasia	Acute and chronic prostatitis	Squamous metaplasia
P-73016N	46.2	+	-	-
B-73023N	49.1	-	+	-
D-73026N	46.4	+	-	-
S-74019N	46.5	+	-	-
M-74021N	34.7	+	-	-
B-74022N	55.0	+	-	-
J-74023N	33.2	+	-	-

Table 2. Prostatic tissue non-competitive in radioimmunoassay compared with histological features

Tissue	Mean % competition	Nodular hyperplasia	Acute and chronic prostatitis	Squamous metaplasia
S-73015N	27.5	+	-	-
T-73018N	11.6	+	-	+
S-73020N	28.1	+	-	-
R-74003N	7.3	+	+	-
L-74006N	24.5	+	+	-
D-74011N	14.2	+	-	-
M-74012N	4.3	+	-	-
T-74016N	19.3	+	-	-
S-74018N	7.1	+	-	-
H-74020N	22.1	+	-	-
M-74025N	14.0	+	-	-
O-74028N	14.8	+	-	+
F-74033N	28.5	+	-	-
B-74040N	10.0	+	-	-

response displacement curve of one BPH protein extract which competed specifically with the ^{125}I-MuLV p30 for binding sites on the FeLV p30 antibody. To demonstrate that this competition was specific, competition assays were run utilising other internal proteins of both murine and leukaemia viruses as competing proteins (not shown). These protein antigens, although from the same viral groups, did not compete with the interspecies p30 antigens utilised in the competition immunoassays.

Prostatic tissue from 21 patients who had clinical BPH have been studied (Tables 1 and 2). A single patient had only "acute and chronic prostatitis" on histological examination without any evidence of nodular hyperplasia or adenocarcinoma. Twenty tissues were diagnosed histologically as nodular hyperplasia and of those, six (30%) were competitive in radioimmunoassay (Table 1). The seventh reactive tissue was from the individual mentioned previously who had only acute and chronic prostatitis. Two other tissues diagnosed pathologically as "nodular hyperplasia with acute and chronic prostatitis" did not compete in the radioimmunoassay (Table 2). There does not appear to be any correlation between results obtained by radioimmunoassay and the histological finding of squamous metaplasia (Tables 1 and 2). Tissues from the two patients diagnosed as squamous metaplasia were non-competitive in the radioimmunoassay.

DISCUSSION

The purified p30 component of murine and feline leukaemia virus was chosen for ^{125}I-labelling in order that the labelled polypeptide

be specific for its antibody. If crude prostatic extract were labelled, only a small portion of the total labelled proteins could possibly react with the antiserum. Furthermore, the concentration of the total reactive protein would vary between specimens depending upon the original amount present as would the efficiency of labelling of individual tissue extracts. By labelling the purified p30 antigen of feline or murine leukaemia virus and allowing known quantities of labelled antigen to compete with tissue extracts for sites on the antiviral globulins, it was possible to quantitate the amount of cross-reacting antigen within the tissue extracts.
A 30% minimum displacement of the labelled antigen in the competition assays was chosen as the limit of non-specific competition, displacement of less than 30% being insufficient to consider a tissue extract positive. Few non-competitive tissues approach 30% competition; likewise, most of the competitive tissues are well above the 30% minimum. Others, using similar competition assays with baboon type C virus rather than murine and feline viruses, have designated a 20% or greater displacement of the labelled test antigen by tissue extract as "antigen-positive." (10)
The data indicates the presence of an oncornavirus-like protein in 30 percent of benign prostatic tissue. The implications of this observation remain unclear. It is possible that proteins other than oncornavirus interspecies antigen were responsible for the observed competition. The tissue may have been contaminated in handling despite aseptic precautions. The assay could be detecting another protein antigenically similar to the viral proteins, thus having the ability to cross-react. Finally, the assay could be detecting "passenger virus" proteins aetiologically unrelated to the occurrence of benign prostatic hyperplasia. Viruses can infect cells and cause no cellular destruction or they may infect cells and produce no cellular alteration until changes occur within the cell's metabolic environment. These studies do not imply viral aetiology of benign prostatic hypertrophy, but they do indicate an association which should be examined further. Oncornavirus genetic information has been detected in a few human tumour cell lines. Birkmayer et al. (3) demonstrated the occurrence of virus-like particles as well as reverse transcriptase activity in metastases of human melanoma. Ilyin et al. (8) have reported an immunological study of a type B oncornavirus isolated of the human cancer cell line HEp2. The virus contained a group specific antigen which cross-reacted with the antigen of the Rhesus monkey oncornavirus type B but did not cross-react with the antigen of the mouse mammary tumour virus, also of type B. No cross-reaction was demonstrated between their virus and the oncornavirus of type C which had been isolated from a human cell line, H22. Here also the question of an aetiological relationship remains unresolved.
The presence in BPH tissue of some antigen related to known RNA tumour viruses has been demonstrated in this communication. Tissue specificity for this antigen is not implied. We have shown that similar antigens are present in other urogenital tissues (9) and others have shown similar antigens in other human tissues, both benign and malignant (10, 12). These findings indicate that human tissues contain the genetic information for proteins analogous to those of one or more type-C oncogenic viruses to other mammalian species. The studies detailed here establish a relationship between antigens related to known RNA tumour viruses and proteins extracted from benign hyperplastic tissue. The question of endogenous oncogene expression with changing hormone milieu must be considered. It is also possible that hyperplastic tissue may be more susceptible to infection by exogenous viruses which invade the urinary tract.
In summary, a sensitive radioimmunoassay using the interspecies antigenic component of feline and murine leukaemia viruses was applied to aqueous extracts of tissue clinically and histologically diagnosed as prostatic nodular hyperplasia. Thirty percent of 20 extracts studied demonstrated competition with viral interspecies antigen for binding sites on the antiviral globulins. These results probably represent the detection of viral polypeptides. The following criteria have been used in the search for a human tumour virus (3):

(1) the presence of an RNA-instructed DNA polymerase,

(2) the presence of high molecular weight (70S) RNA,

(3) demonstration of virus-specific nucleic acid sequences and

(4) antigens related to known RNA tumour viruses.

Evidence for the fourth criterion has been demonstrated in this communication using human nodular hyperplastic tissue. Further investigation of prostatic nodular hyperplasia tissues is continuing. The tissues will be examined for evidence of competition by radioimmunoassay, for reverse transcriptase activity, and for visualisation of C-type virus like particles by electron microscopy.

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