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Autoantibodies to Prostasomes as New Markers for Prostate Cancer

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ABSTRACT

Prostate cancer is one of the leading causes of cancer-related death among men. Given the varying clinical course and the long natural history of the disease, it is important to have good diagnostic and prognostic markers. Prostate specific antigen (PSA) is currently the best marker for the detection of prostate cancer, but in many cases it does not reveal whether metastases have appeared. Since metastases of prostate cancer release prostasomes, which are immunogenic secretory granules of both normal and neoplastic prostate cells, we checked whether anti-prostasome antibodies will appear when the cancer is metastasing. In a pilot study, all 13 patients with serum PSA between 50-500 μg/L had anti-prostasome antibodies, while 39 healthy controls with low PSA values showed background values. There was no overlapping, i.e. the upper range value of controls did not reach the lower range value of patients.

INTRODUCTION

Although diagnosis and therapy of localized prostate cancer have made progress, there is still need for a method to determine whether neoplastic cells have disseminated to form micrometastases. Since improved assays to identify this group of patients would influence the therapy of the disease considerably, various methods have been tried to detect occult metastases. For instance, both immunohistochemical staining using different markers (5, 12, 17) and molecular biology using the polymerase chain reaction (1, 9) have been attempted to find neoplastic cells in the bone marrow, but none of the diagnostic techniques designed have been applied in clinical routine as yet. Therefore, we tested a new approach, namely to detect whether neoplastic cells, which have emerged outside the prostate barrier, will induce antibody production to prostasomes, that is, a secretory product of the prostate cells.

Prostasomes, which are small (40-500 nm) granules or vesicles, are synthetized in the prostate epithelial cells and are released with the prostate secretions into the prostate gland ducts (8). Normally, the prostate cells and the excretory ducts form, immunologically seen, a closed system and the balance among the various immune cells impedes destructive immunological reactions in the prostate. Since the prostate antigens are hidden or do not appear until puberty, the immunological system regards them as foreign and may create autoantibodies against them if they escape outside the immunological barriers. Similar situations hold true for, among other organs, the testis. We assumed that also prostate adenocarcinoma cells, which can leave the prostate and settle as prostasome-producing metastases (16), should be able to evoke antibodies to prostasomes.

Therefore, in a pilot study we checked the presence of anti-prostasome antibodies in sera from 13 patients having prostate cancer with elevated levels of prostate specific antigen (PSA) and from 39 control patients. We detected serum autoantibodies to prostasomes but not to PSA in all the diseased patients. This report informs about the study.

MATERIALS AND METHODS

Serum samples

Serum samples from 13 men with PAD verified prostate cancer and PSA levels between 50-500 μg/L were investigated. Five patients were known to have metastases, while no metastases were demonstrated clinically in the remaining 8 ones. As controls we used sera from healthy blood donors, 19 men and 20 women, age 20 - 40 years, with PSA levels < 2.5 μg/L.

Anti-prostasome ELISA

An enzyme-linked immunosorbent assay (ELISA) was used to detect anti-prostasome antibodies in serum. Plates (F96, Polysorp, Nunc) were coated with 4 μg purified prostasomes, isolated from human prostatic tissue, diluted in 100 mmol/L NaHCO3, pH 9.5, (coating buffer) for 2 h at 37° C. The plates were then washed and blocked for 1 h at 37° C with the coating buffer containing 3 % BSA. After blocking, the plates were washed 3 times with 200 μL phosphate buffered saline containing 0.1 % Tween (PBS-T) and incubated with 200 μL patient or control serum, diluted 1:50 in PBS, for 2 h at 37° C. After 3 new washes with 200 μL PBS -T, 100 μL goat-antihuman IgG horse radish peroxidase (HRP) conjugated antibodies, diluted 1:1,000 in PBS, were added and incubated for 1 h at room temperature.

The plates were washed 3 times with 200 µL PBS-T and incubated with substrate (tetramethyl benzidine, Zymed Laboratories, Inc., CA, USA) for 15 min at room temperature while being protected from light. The reaction was stopped by adding 50 µL 1.8 mol/L sulphuric acid. The absorbance was measured at 450 nm in an ELISA reader (SPECTRA Max 250, Molecular Devices, Sunnyvale, CA, USA).

Polyacrylamide gel electrophoresis and immunoblotting

Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS PAGE) and immunoblotting were performed with Bio-Rad systems according to the instructions of the manufacturer. Purified prostasomes isolated from human prostatic tissue were loaded on a 12-14% polyacrylamide gel. For immunoblotting, the proteins were electro-transferred to nitrocellulose membrane. The membranes were thereafter blocked for 1 h with 5% skim milk in PBS. For visualisation of the proteins, the membranes were incubated overnight with patient serum diluted 1:50 in PBS. The following day, the membranes were incubated for 1 h with biotinylated anti-human IgG (Zymed Laboratories Inc.) and thereafter with Streptavidin alkaline phosphatase (Zymed Laboratories Inc.) for 1 h with 3 intermediate washings in TBS. The visualisation was made by NBT/BCIP tablets (Boehringer Mannheim, Mannheim, Germany).

RESULTS

All patients tested had detectable levels of autoantibodies to prostasomes, while all controls showed background values without overlapping. There was no significant difference between males and females in the control group. The absorbance interval for the control group was 0.03-0.15 (0.097(0.04, mean (S.D.) measured at 450 nm, whereas all patients had absorbance values that were higher than the controls, 0.23-0.34 (0.278(0.03) (Figure 1).

Western blotting with patient sera after SDS-PAGE separation of prostasomes revealed protein bands with the molecular weights of 8 and 60 kDa (Figure 2). The pattern differed between individual patients. No band was visible in the molecular range between 8 to60 kDa, excluding the possibility of reactivity of the patient sera with PSA (approximately 30 kDa).

DISCUSSION

All the 13 prostate diseased patients had a PSA value above 50 µg/L, which is judged to indicate that they were carrying a metastasing cancer. Our present results showed that they all

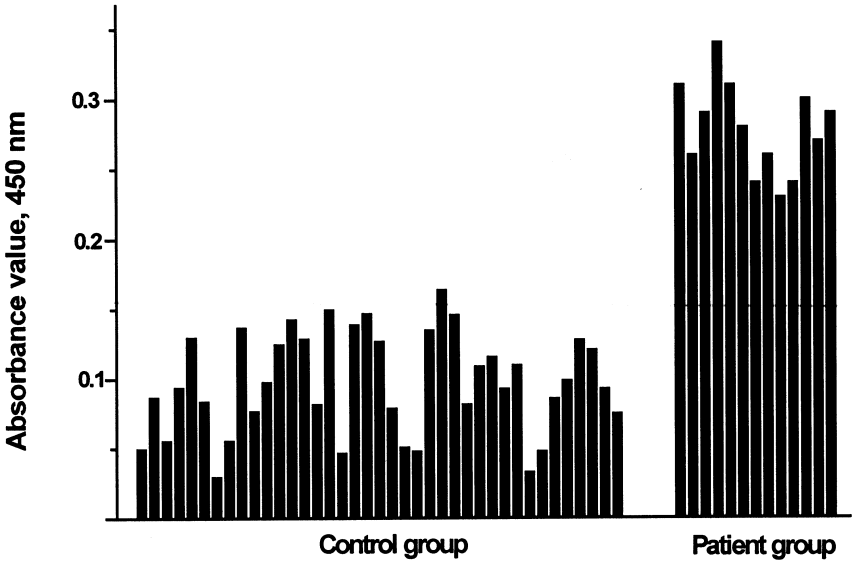

Fig. 1. Anti-prostasome ELISA absorbance values of 39 controls and 13 men with prostate cancer.

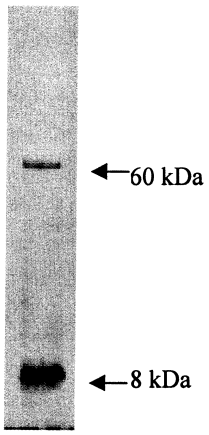

Fig. 2. Prostasomes separated on SDS-PAGE and electroblotted to nitrocellulose membrane. Antigen was demonstrated with patients´s serum.

harboured serum anti-prostasome antibodies which according to our hypothesis signifies metastases of prostate cancer. All of the controls demonstrated serum samples with background values of anti-prostasome antibodies. Thus, the pilot study suggests that prostasomes, secreted from neoplastic prostate cells which have escaped outside the prostate borders, are able to raise autoantibodies. At present, we are therefore evaluating the possibility to use autoantibodies against prostasomes as a diagnostic test for a prostate cancer. Autoantibodies to tumour-associated antigens have previously been shown to be useful as indicators of tumour presence and as prognostic markers. For instance, mutations in the p53 gene result in an overexpression of p53 in a tumour. The concentration is too low to be recognized in serum, but some patients with various carcinomas mount a detectable humoral immune response against the abnormal p53 protein. For instance, serum anti-p53 antibodies were detected in patients with ovarian cancer (20) and lung cancer (4, 13). In addition, these autoantibodies provided prognostic information.

The immune responses observed in patients with prostate cancer also open possibilities for immunotherapy with anti-prostasome antibodies (6, 15). Several vaccination studies have addressed methods to evoke an immune response against specific tumour antigens (11, 14, 18, 19). It may thus be interesting to correlate the response-pattern of autoantibodies from patients with prostate cancer with the progress of the disease, since this may aid the development of new vaccination procedures.

Other uro-genital affections, which have the ability to break immunological barriers, have been suggested to evoke autoimmune responses, among them, non-bacterial prostatitis (2, 7). Further, affections of the Skene´s glands, which are the female homology to the prostate and located around the female urethra, can initiate an autoimmune response and perhaps constitute a component of the so-called vulvodynia or female urethral syndrome (10). Interestingly, also some patients with immunological infertility were found to have developed anti-prostasome antibodies (3), probably due to the coat of prostasomes which the sperm cells are carrying (21). Therefore, it seems that the prostasomes could be a frequently active immunogen of various uro-genital diseases.

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