ANTICANCER RESEARCH *25*: 4399-4402 (2005)

Antiprostasome Antibody Titres in Benign and Malignant Prostate Disease

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Abstract. *Background: Prostasomes are secretory granules synthesized, stored and secreted by normal and neoplastic human prostate epithelial cells and by prostate cancer metastasis. Prostasomes are postulated to be shed into the blood circulation in prostate cancer patients, where they may cause an immune response. Materials and Methods: An antiprostasome antibody ELISA was developed and used to measure serum antibody titres in 81 males with prostate cancer or benign prostate hyperplasia. Results: Patients with biopsy-verified prostate cancer had significantly higher antibody titres [p=0.019 for the dominant tumour expression (first Gleason parameter) and p=0.022 for the dominant and non-dominant form (first and second Gleason parameters)] than individuals with benign prostate hyperplasia or other benign prostate disorders. No correlation existed between antibody titres and PSA values. Conclusion: These results indicate that the antiprostasome antibody titre in serum may be a novel prostate cancer marker not replacing, but rather supplementing, PSA.*

A wide variety of morphological and molecular markers have been studied for their ability to predict outcome in prostate cancer (1-4). Conventional morphology-driven measures have included tumour grade, volume and pathological stage. Although a number of molecular markers have been proposed for their potential clinical utility (1), their use has been hampered by suspicions regarding lack of specificity and sensitivity of the marker in question. Other shortcomings have been the limited availability of tissue and, due to the inherent heterogeneity of the prostate cancer specimen, doubts about the relevance of the results (2). We wanted to study a new and simplified concept in this matter, the appearance of antiprostasome antibodies in the serum of patients with prostate tumours and their possible relationships to other tumour expressions.

Prostasomes are secretory granules and vesicles that are produced, stored and released into the ducts by the glandular epithelial cells of the prostate (5-7). Intracellularly, the prostasomes are present as inclusions in storage vesicles originating from the Golgi membranes by a budding process (5, 8). The prostasomes, intracellularly encased in the storage vesicles, are then released by exocytosis into the acinar lumen of the gland after fusion between the membrane surrounding the storage vesicle and the plasma membrane of the prostate epithelial cell (5, 8). Prostasomes are not only the secretory product of the normal prostate gland, but also of prostate cancer of different grades (8-10). The prostasomes are membrane-surrounded by a lipid bilayered structure in their extracellular appearance (5, 11). The membrane has an unusually high cholesterol / phospholipid ratio affording the prostasomes very stable properties (12, 13). The protein content is composite, with at least 200 membrane protein entities (14, 15).

It is well known that malignant prostate cancer cells are spread by the blood circulation (16-18). Since the prostasomes are very small (mean diameter 150 nm) and continuously released, we assume that the discharge of the very small prostasomes into the blood stream is an earlier event than that of the large prostate cancer cells. Numerous studies suggest that prostate cancer cells can be rendered immunogenic and induce an immune response (19-23). Analogously, a corresponding response by prostasomes would be anticipated. A previous pilot study proved the presence of serum autoantibodies against prostasomes in patients having prostate cancer and a serum PSA value of 50 μg/L or more (24). Furthermore, an inverse relationship

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Key Words: Autoantibodies, ELISA, prostasome, prostate cancer, prostate specific antigen.

was established between the antiprostasome autoantibody titre in the serum and the outcome of prostate cancer in terms of metastases to lymph nodes and the skeleton (25).

The aim of the present study was to examine the antiprostasome antibody response in the serum of patients with benign prostatic hyperplasia and highly differentiated prostate cancer.

Materials and Methods

Study population. Eighty-one patients, with defined diagnosis, were included in the study; 45 of these patients had benign prostatic hyperplasia (BPH) including 7 patients with an additional chronic prostatitis. Thirty-one patients had prostate cancer with Gleason grading from G2+2 to G5+4. The remaining 5 patients had diagnoses of benign tumour, inflammation, chronic prostatitis, PIN and dysplasia.

Sample collection and medical history. Blood samples were drawn from an antecubital vein and collected in Vacutainer® tubes without additives (Becton Dickinson, Franklin Lakes, NJ, USA). The serum samples were immediately frozen at –70˚C until analysis. The prostate cancer diagnosis was settled by histological examination of the prostate biopsies.

Prostasome preparation. Seminal prostasomes were isolated and purified as reported earlier (15). In brief: semen samples were allowed to liquefy for 30-45 min and were then centrifuged for 20 min at 1,000 xg and 22˚C to separate spermatozoa and other cells from the seminal plasma, which were pooled (12-15 samples) and ultracentrifuged at 10,000 xg for 15 min at 4˚C to remove possible cell debris. The supernatant was subsequently subjected to another ultracentrifugation for 2 h at 100,000 xg and 4˚C to pellet the prostasomes. The prostasomes were resuspended in 30 mmol/L Tris-HCl, containing 130 mmol/L NaCl, pH 7.6 (isotonic Tris-HCl buffer). This prostasome suspension was further purified on a Sephadex G 200 column (Amersham Biosciences, Uppsala, Sweden), equilibrated with the isotonic Tris-HCl buffer, to separate the prostasomes from an amorphous substance (5). The eluant was the isotonic Tris-HCl buffer, and the eluate was monitored at 260 and 280 nm. Those fractions (5-12), with initially elevated UV absorbances, were collected and analysed for aminopeptidase N activity, a marker enzyme for prostasomes (15). Ultraviolet-absorbing fractions with high aminopeptidase N activity were pooled and ultracentrifuged at 100,000 xg for 2 h and 4˚C. The pellet representing the prostasomes was resuspended in the isotonic Tris-HCl buffer and adjusted to a protein concentration of 2 mg/mL using a Protein Assay ESL method (Roche Diagnostics, Mannheim, Germany).

Antiprostasome ELISA. Microtitre plates (F96, Polysorp, Nunc) coated with 4 μg of purified seminal prostasomes and positive and negative standards were obtained from Immunsystem AB (Uppsala, Sweden). The plates were incubated with 100 μL of serum samples, positive and negative standards (diluted 1:50 in PBS) for 2 h at 37˚C. After 3 new washes with 200 μL PBS-T, 100 μL goat anti-human IgG horseradish peroxidase (HRP) -conjugated antibodies (Zymed Laboratories, Inc., CA, USA), diluted 1:1,000 in PBS, were added and incubated for 1 h at 22˚C. The plates were washed 3 times with 200 μL PBS-T and incubated with substrate (tetramethyl benzidine, Zymed Laboratories, Inc.) for 15 min at 22˚C while being protected from light. The reaction was stopped by adding 50 μL of 1.8 mol/L sulphuric acid. The absorbance was measured at 450 nm in an ELISA reader system (SPECTRA Max 250, Molecular Devices, Sunnyvale, CA, USA). The titre of the patient sera was calculated in relation to the standards. The value assigned for the positive standard was 1.0 and for the negative standard 0 (sample value = (A450 of patient sample – A450 of negative standard)/(A450 of positive standard – A450 of negative standard). The standards were used to reduce inherent absorbance differences between different microtitre plates. The standards were chosen based on previous results (24).

Table I. *Correlation between antiprostasome ELISA titre and age, prostate volume, adenoma volume, PSA value and Gleason grading of the dominant tumour expression (first Gleason parameter) for individual patients and a combination of the dominant and non-dominant form (the sum of the first Gleason parameter and 50% of the second).*

Correlations	Spearman	
	R	*p*-level
ELISA and age	-0.075	0.512
ELISA and prostate volume	0.032	0.817
ELISA and adenoma volume	0.003	0.981
ELISA and PSA	0.122	0.268
ELISA and Gleason grading (first value)	0.260	0.019
ELISA and Gleason grading (both values)	0.253	0.022

Statistical calculations. Statistical analysis was performed with Spearman Rank correlation utilizing Statistica 4.5 (Statsoft Inc., Tulsa, OK, USA) and Excel 97 (Microsoft Corp., Seattle, WA, USA). For statistical analysis the value for the Gleason grading of the dominant tumour expression (first parameter according to Gleason) was used or a combination of the first parameter and 50% of the value of the second parameter according to Gleason. Dysplasia and benign tumour were assigned the value 0.5, and all benign states were assigned the value 0. *P*-values < 0.05 were regarded as statistically significant throughout the study.

Results

The mean age of patients with benign prostatic hyperplasia was 66 years (range 48-79 years) and that of patients with prostate cancer 68 years (range 53-89 years). Forty-eight patients had benign prostatic hyperplasia displaying a mean serum PSA value of 9.0 μg/L (range 0.00-27) and a mean antiprostasome antibody titre of 0.30 (range 0.02-1.3). Corresponding figures of patients with prostate cancer, a majority of whom (58%) had highly-differentiated tumours (Gleason score ≤6), were 27.6 μg/L (range 0.71-174) and 0.41 (range 0.05-1.5), respectively. From Table I it can be seen that no significant relationship existed between the antiprostasome antibody titre and patient age, prostate and adenoma volumes, or PSA values. However, a significant correlation was apparent between the antiprostasome antibody titre and tumour grading, as illustrated in Figure 1.

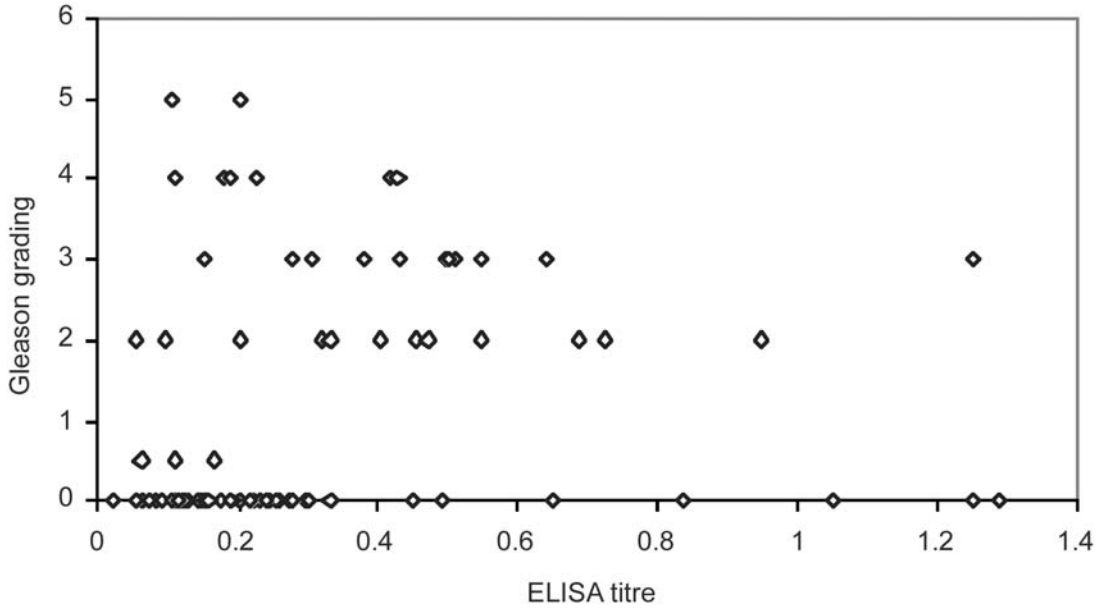

Figure 1. *Correlation between antiprostasome ELISA titre and Gleason grading of the dominant tumour expression (first Gleason parameter) for individual patients.*

Discussion

Prostate biopsies are currently the foundation for diagnosis of prostate cancer. This method is, however, limited by several technical problems. It is difficult to obtain representative materials due to the obstacles, even for the experienced, associated with obtaining material from a small tumour and to the inherent heterogeneity of the prostate cancer specimen (2). The recommendation of multiple biopsies (>10-15) emphasizes the problem. Multiple prostate biopsies are expensive to perform and are also painful for the patient. It is thus highly desirable to find serum or plasma markers that could, in a simple way, provide similar or better information than the biopsies. The most widely used marker for detection of prostate cancer is serum prostate-specific antigen (PSA), measured as total PSA or in combinations that distinguish between free and complex-bound PSA.

The use of PSA for screening of prostate cancer is hampered by the sensitivity of PSA and the fact that it is an organ-specific marker rather than a cancer-specific marker. Many of the tumours detected by PSA do not influence the life expectancy of the carrier. This is due to the natural history of prostate cancer which is highly variable, and men are much more likely to die with, rather than because of, prostate cancer (26). The rate of latent prostate cancer is high – 50% for men in their fifties and more than 75% for men older than 85 years (27). Although the life-time risk of having microscopic (latent) prostate cancer for a man 50 years of age is 42%, the risk of dying from prostate cancer is about 3% (26). The problem of distinguishing between prostate cancer that will kill the patient and the one that will not affect the life expectancy of the individual patient emphasizes the need for new markers.

The usefulness of PSA would be greatly increased if it could be combined with a marker that could provide information about the degree of aggressiveness and tumour progression for the individual patient. Such combined biochemical markers would improve the treatment benefit/adverse effects ratio and thus increase the efficiency of prostate cancer screening programmes.

In the present study, the increased antiprostasome antibody titre determined in serum by our sensitive ELISA technique correlated significantly with the Gleason score among the patients with predominantly highly-differentiated prostate cancer. This appears to be an unconventional attempt to link the serum antiprostasome antibody titre with the degree of differentiation and, thus, with the outcome of prostate cancer, given that a higher degree of differentiation has a more favourable outcome. The use of antiprostasome antibodies as markers for prostate cancer is a new concept and could be an important supplement to PSA in the future. The concept that antiprostasome antibodies could provide prognostic information is in agreement with the initiation of recent vaccination programmes aimed at improving survival of prostate cancer by immunising with prostate cancer antigens (28). We have recently identified clusterin as an autoantigen in prostasomes (15, 29). Clusterin has a wide array of functions, including roles in reproduction, lipid transport, tissue

remodelling and apoptosis (30-32), and an autoantibody to clusterin may thus inhibit these functions. The implication of this is that there may be functional differences and differences in predictive value between antiprostasome antibodies directed against different antigens. A recent proteomic analysis of human prostasomes, applying microcapillary liquid chromatography-tandem mass spectrometry, revealed that they contain at least 139 proteins subdivided into 6 categories (14), including enzymes (35%), transport/structural proteins (19%), GTP proteins (14%), chaperone proteins (6%), signal transduction proteins (17%) and novel proteins (9%). The identification of which of these antigens evokes an autoantibody response allows for the development of ELISA for specific prostasomal autoantigens. Such assays may further increase the prognostic information provided by antiprostasome antibodies.

Acknowledgements

This study was financially supported by the Swedish Cancer Society.

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Received June 14, 2005
Accepted September 2, 2005