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Gammopathy associated with advanced prostate carcinoma

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Abstract As proteinuria was found to be common in patients with prostate cancer, the possible presence of elevated urinary immunoglobulin (Ig) levels was investigated. First morning urine samples from 30 patients with androgen-dependent (AD) and 43 patients with androgen-independent (AI) prostate cancer were tested. A sensitive, solid-phase radioimmunoassay and polyclonal antibody interacting with IgG, IgA and IgM was used to screen urine samples. Compared with 15 normal, age-matched, healthy subjects, urinary Ig levels were elevated in 10 of 30 (33%) patients with AD prostate cancer and in 24 of 43 (56%) patients with AI prostate cancer. In the latter group, five of seven (71%) patients with prostatic SCC had elevated urinary Igs. Further analysis of ten urine samples containing increased urinary Ig levels, using antibodies specific for each heavy chain (gamma, alpha and mu) as well as each light chain (kappa and lambda), indicated that patients with a high or moderate elevation in Ig levels had polyclonal gammopathy, whereas those with a low increase in urinary Igs had monoclonal gammopathy of the IgG class. These results indicate, for the first time, a high frequency of an abnormal increase in Ig levels in patients with advanced prostatic carcinoma.

Key words Gammopathy · Prostate cancer · Urinary immunoglobulins · Monoclonal · Polyclonal · Androgen-independent

Monoclonal gammopathy is defined as an abnormal increase in monoclonal protein (M-protein or paraprotein), consisting of two heavy immunoglobulin (Ig) chains of the same class and subclass and two light chains of the same type [6]. In contrast, a polyclonal Ig increase consists of one or more heavy-chain classes and/or both light-chain types [6]. In addition to multiple myeloma and its variants, monoclonal Igs have been found with significant frequency in chronic lymphocytic leukemia and a variety of lymphomas [3, 5–8]. Paraproteins have also been reported in certain solid malignancies, but with low frequency [10].

In preliminary work in our laboratory, to determine urinary levels of bombesin/gastrin-releasing peptide (GRP) [4], we found that proteinuria was common in patients with prostate cancer. We therefore investigated the presence of paraproteins in the urine samples. Analysis of serum and urine for M-protein requires a method to detect the presence of immunoglobulins and a specific assay to identify it according to its heavy-chain class and light-chain type [7]. We developed radioimmunoassays (RIAs) to meet these requirements. Our results described below indicate a high frequency of urinary Ig elevation in patients with advanced prostate carcinoma.

Materials and methods

First morning urine specimens from patients with prostate cancer were assayed. Urine samples were centrifuged in 15-ml sterile tubes at 800 *g* for 10 min, and the supernatant was divided into 1-ml aliquots in Eppendorf tubes. Each aliquot was preserved by the addition of acetic acid to a final concentration of 2 N and stored in a − 20 °C freezer until used in the RIAs.

The following RIA procedure was used: 2–20 µl acid-treated urine was diluted to 0.5 ml with 0.1 M bicarbonate buffer, pH 9.6, and placed in immunoassay tubes (Star tubes, Nunc, Denmark). After shaking overnight at room temperature (RT), urine was aspirated and tubes blocked by 1–2 h treatment with 0.7 ml 2% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) (pH 7.4) followed by a 2- to 4-h treatment at RT with 0.5 ml primary antibody diluted 1:1000 in 0.5% BSA, 0.05% Tween-20, PBS (assay buffer). After a 5-min wash with assay buffer, bound antibody was detected by 2–3 h treatment at RT with ^{125}I-protein G (Amersham, Ill., USA), 3×10^5 cpm/0.5 ml per tube in assay buffer. After

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washing with 0.7 ml assay, buffer tubes were counted in a gamma counter.

All primary antibodies were from Chemicon International (Temecula, Calif., USA). Antisera had been prepared against human Igs in goat. The antiserum used for the initial screening for the presence of Igs in urine cross-reacted with human gamma (G), alpha (A) and mu (M) heavy chains. The antisera used for the identification of Ig class were specific for each heavy-chain class and for each light-chain type, kappa (free and bound forms) and lambda (free and bound forms). Serum prostate-specific antigen (PSA) was measured using an automated, fluorescence-based, two-site, immmunoenzymetric assay (TOSOH, Calif., USA).

Results

Low levels of immunoglobulins were found in the urine of normal healthy subjects (Fig. 1). Compared with these age-matched normal controls, 46% (34/73) of patients with advanced prostatic carcinoma showed an elevation in urinary immunoglobulins. Patients with active cancer who had not received hormonal therapy and had normal serum testosterone levels were considered androgen dependent. Patients with an objective

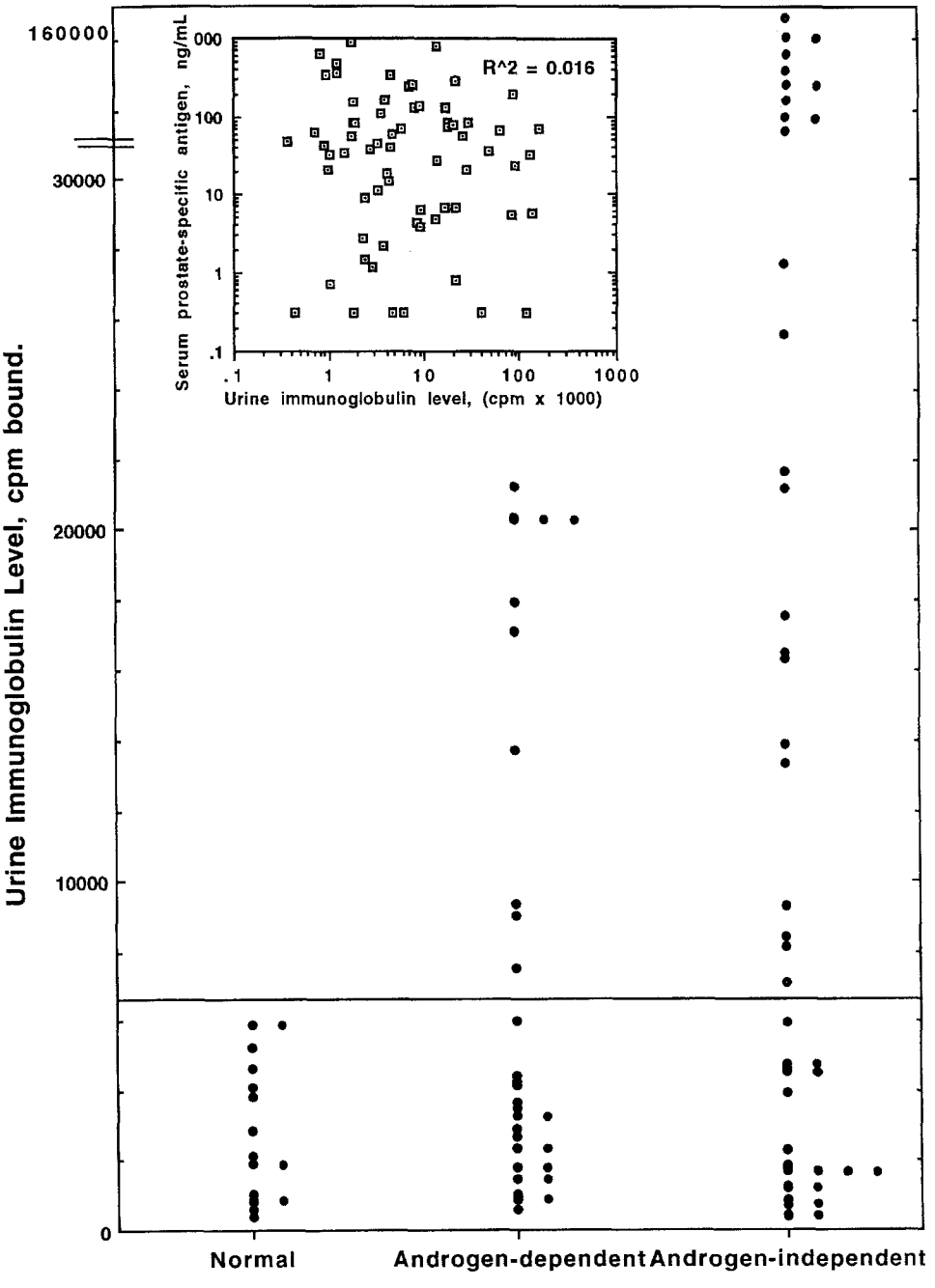

Fig. 1 Urinary immunoglobulin levels in patients with prostatic carcinoma. In normal, healthy subjects the average counts were 2825 ± 1837 (mean ± SD). Upper limit of normal (mean ± 2SDs) is indicated. *Inset* shows lack of correlation between serum PSA levels and urinary immunoglobulin levels in patients with prostatic carcinoma

progression of the disease in the presence of castrate levels of serum testosterone (<50 ng/dl) were considered androgen independent. In patients with androgen-dependent disease, 33% (10/30) of patients had elevated urine Igs (Fig. 1). In this group of 30 patients, 13% (1/8) of stage C, 38% (3/8) of stage D1 and 43% (6/14) of stage D2 patients showed an increase. Thus, the frequency of urine Ig elevation increases with metastasis.

An elevation in urinary Ig levels was observed in 56% (24/43) of patients with androgen-independent prostate carcinoma (Fig. 1). In this group, 71% (5/7) of patients with prostatic small cell carcinoma (SCC) had an Ig increase. Morphological criteria for small cell carcinoma (small malignant cells, measuring about twice the diameter of normal lymphocytes, possessing a pyknotic round to oval nucleus, evenly distributed chromatin, inconspicuous nucleoli and scanty cytoplasm) established for lung tumors by the World Health Organization [12] were fulfilled in these seven cases. Prostatic SCC was characterized by low serum PSA, hormonal resistance, rapid metastatic spread and shortened survival [1]. Of the 36 patients with androgen-independent adenocarcinoma, 2 of 3 patients with stage D1 and 17 of 33 patients with stage D2 disease had elevated urinary Igs. Thus, 53% (19/36) of patients with androgen-independent adenocarcinoma had elevated urinary Igs.

While low to moderate Ig elevations occurred in patients with androgen-dependent disease, high elevations were found in several patients with androgen-independent prostate cancer (Fig. 1). Ten urine samples containing abnormal Ig levels were further analyzed. Results are shown in Table 1. In four urine samples with a low elevation, monoclonal gammopathy of the IgG class was found. In two urine samples with a moderate Ig increase and four samples with a high increase, polyclonal gammopathy was detected. There was no correlation between serum PSA values and urine Ig levels (Fig. 1, inset). Also, there was no correlation between patient age and urinary Ig level (Fig. 2).

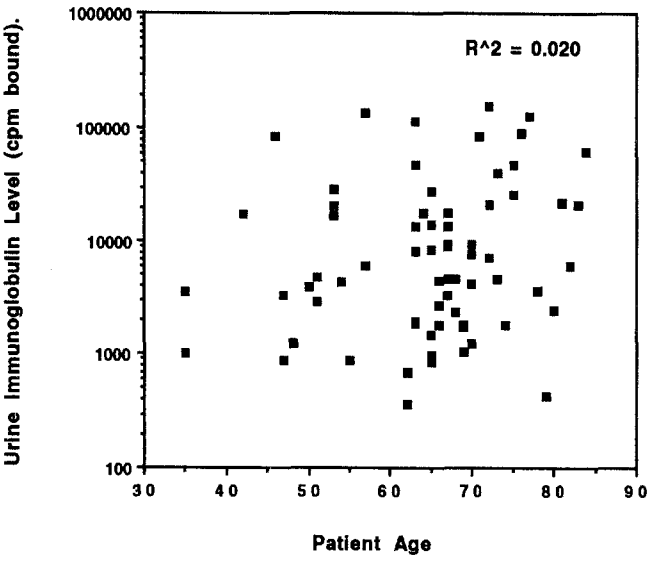

Fig. 2 Lack of correlation between age of prostate cancer patients and urinary immunoglobulin levels

Information on histological grade of the primary tumor was available for 55 patients. Elevation in urinary Igs were found in 2 of 2 patients with a Gleason score of four, 0 of 3 patients with a score of five, 2 of 6 with a score of six, 6 of 10 with a score of seven, 7 of 12 with a score of eight, 4 of 18 with a score of nine and 3 of 4 with a score of ten. Thus, all tumors with high Gleason scores were not accompanied by elevated Igs. However, all patients showing moderate to high elevations in urinary Ig levels had tumors with Gleason scores of 7 or higher.

Discussion

Monoclonal immunoglobulins in the serum and urine are the earliest forms of tumor markers [5]. These are generally identified by a monoclonal peak on serum or urine electrophoresis and confirmed by

Table 1 Type of gammopathy in 10 patients with elevated levels of urinary immunoglobulins (Ig)

Hormonal status	Ig elevation	Heavy-chain class	Light-chain class	Type of gammopathy
AI	Low	IgG	Kappa	Monoclonal
AI	Moderate	IgG, IgM	Kappa, lambda	Polyclonal
AI	Moderate	IgG	Kappa, lambda	Polyclonal
AI	High	IgG, IgM, IgA	Kappa, lambda	Polyclonal
AI	High	IgG, IgM, IgA	Kappa, lambda	Polyclonal
AI	High	IgG, IgM, IgA	Kappa, lambda	Polyclonal
AI	High	IgG, IgA	Kappa, lambda	Polyclonal
AI	Low	IgG	Kappa	Monoclonal
AI	Low	IgG	Kappa	Monoclonal
AD	Low	IgG	Kappa	Monoclonal

(*AI*, androgen-independent; *AD*, androgen-dependent)

immunoelectrophoresis and/or immunofixation. While increases in paraproteins have been reported in hematological malignancies using these techniques, no significant increase has been established for solid malignancies [8]. In a study with 807 patients representing a variety of solid malignant neoplasms including prostate cancer, there appeared to be no evidence of an increased frequency of monoclonal proteins compared with the normal adult population [10]. However, using a sensitive method (radioimmunoassay), we find a high frequency of gammopathy in patients with advanced prostatic carcinoma.

Nearly half of the 73 patients tested had elevated urinary Ig levels. High frequencies were found in patients with metastatic and androgen-independent disease. Hence, urinary Ig levels increased with tumor progression. In patients with low elevations of Igs, monoclonal gammopathy of the IgG class occurred, whereas those with high elevations had polyclonal gammopathy involving IgM and IgA. Compared with IgG, myeloma patients with IgA gammopathy have a shorter remission duration and shorter survival [5]. Most patients with SCC of the prostate had a high elevation in paraproteins. Prostatic SCC is predictive of a poor outcome [1]. Further studies are needed to determine the possible use of this tumor marker as a prognostic factor and in monitoring the therapy of patients with prostate cancer. Use of this sensitive radioimmunoassay technique may also reveal a higher frequency of paraproteins in other solid malignancies.

The incidence of monoclonal proteins is thought to increase with advancing age [8]. We found no correlation between age and urinary Ig level in this series. Also, there was no correlation between serum PSA and urine Ig levels. Two cases of prostatic cancer accompanied by monoclonal gammopathy have been recently reported [11]. Both cases presented with IgG-type hyperimmunoglobulinemia and both had multiple bone metastases [11]. In another recent study of 44 patients with different prostatic lesions, serum IgA levels showed stage dependence in prostatic carcinoma, being more raised in advanced malignancy (stages C and D) than in localized ones (stage B) [2]. In addition, the serum level of immunoglobulin-binding factor (IgBF) measured by RIA was recently found to be elevated in patients with prostatic carcinoma [9].

Several factors have been implicated in the pathogenesis of gammopathy. Repeated antigenic stimulation of the reticuloendothelial system, genetic susceptibility for the development of plasma cell dyscrasia in patients with a positive family history, Epstein-Barr virus, lymphoid growth factors such as IL-6, impairment of T-cell function and lack of suppression of B cells by T cells among others have been suggested to play an etiologic role in gammopathy (reviewed in ref. [8]). The etiology of immunoglobulin rise in prostatic carcinoma is unclear at the present time and requires further investigation.

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