10. M. Baggiolini, C. De Duve, P. L. Masson, et al., J. Exp. Med., 131, 559 (1970).
11. W. R. Van der Geld, T. Feltkamp and H. I. Oosterhuis, Proc. Soc. Exp. Biol. (New York), 115, 782 (1964).
12. I. M. Lyampert, L. V. Beletskaya, N. A. Borodiyuk, et al., Immunology, 131, 47 (1976).
13. P. L. Masson, J. F. Heremans, and C. De Duve, Clin. Chim. Acta, 14, 735 (1966).
14. P. L. Masson, J. F. Heremans, and E. Schonne, J. Exp. Med., 130, 643 (1969).

ORGAN-SPECIFIC BETA-GLOBULIN OF THE HUMAN PROSTATE DURING TUMOR GROWTH

A. A. Nikolaev, A. V. Afanas'eva, and Yu. Kh. Khairullin

UDC 616.65-006-008.939.624

KEY WORDS: prostate gland; tumor; organ-specific antigen.

The study of the antigenic structure of human organs and tissues is an urgent problem at the present time because of the needs of medical practice and, in particular, of oncology. The characteristics of organ-specific antigens occupy a central position in this problem because, as specific markers of a tissue, they enable the degree of differentiation, the functional maturity and, in some cases, commencing malignant transformation of a tissue to be determined.

The study of the antigenic structure of the human prostate has so far been confined mainly to investigation of only one antigen, namely organ-specific acid phosphatase, the physicochemical properties, structure, and diagnostic importance of which have been characterized in detail [7-9].

While engaged on the study of the antigenic structure of the prostate, besides acid phosphatase we have also identified another organ-specific antigen, which does not possess phosphatase activity and migrates during immunoelectrophoresis in agar in the beta-globulin zone.

The object of this investigation was to study the immunochemical and physicochemical properties of prostatic organ-specific beta-globulin, its tissue localization, and its behavior during tumor growth.

EXPERIMENTAL METHOD

Saline extracts of fetal, definitive, and tumor tissues of the prostate, prepared in Tris-glycine buffer, pH 8.6, in the ratio of 1:3 (w/v), were used. The control group consisted of extracts of fetal and definitive tissues of various human organs prepared in the same way.

Prostatic beta-globulin (PBG) in the tissues was identified by immunoelectrophoresis [1] and double immunodiffusion in agar [5]. The PBG concentration in the tissues and biological fluids was determined by immunodiffusion titration in agar with a standard test system, the sensitivity of which was 0.3 mg %.

PBG isolated from a saline extract of normal prostate and purified by the writers' own method,* was used as the test antigen.

The test antisera were obtained by immunizing rabbits in the usual way both with pure PBG and with saline extracts of definitive prostate mixed with Freund's complete adjuvant. Only monospecific antisera were used in the work; polyvalent antisera were absorbed with

*Awarded Branch Innovation Certificate No. 0-933 dated December 22, 1978.

Department of Biochemistry and Department of Pathological Anatomy, Astrakhan' Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 11, pp. 583-586, November, 1980. Original article submitted December 14, 1979.

0007-4888/80/9011-1565$07.50

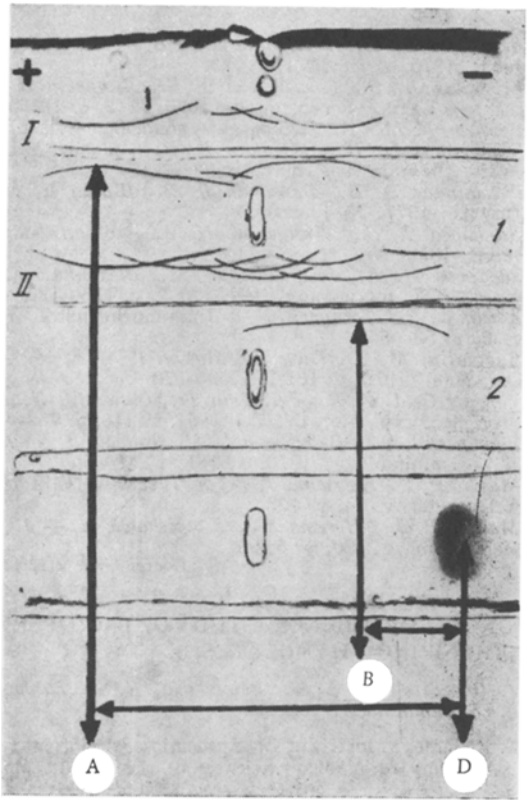

Fig. 1

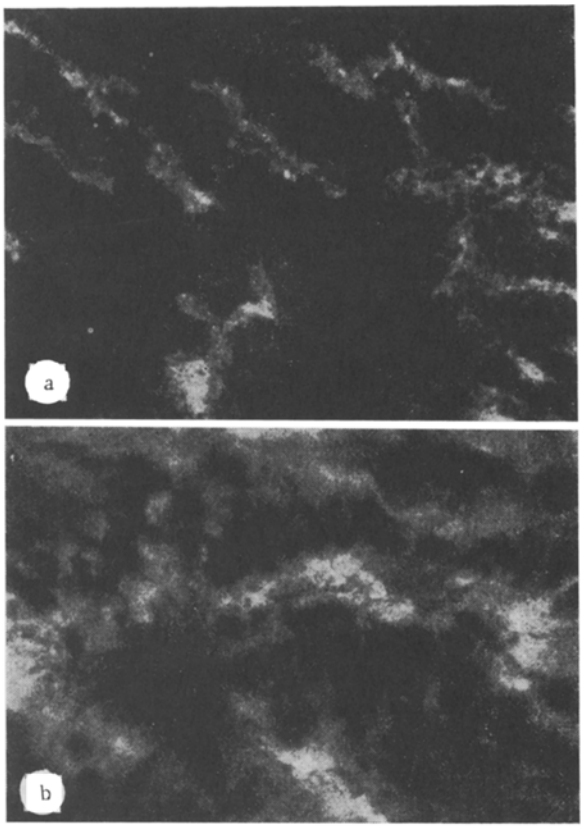

Fig. 2

Fig. 1. Relative electrophoretic mobility of PBG. 1) Extract of normal prostate; 2) purified preparation of PBG; I) antiserum against human blood serum proteins; II) antiserum against extract of normal prostate. D) Dextran; A–D) mobility of human albumin; B–D) mobility of PBG.

Fig. 2. Photomicrograph of the prostate. Indirect immunofluorescence method: a) general appearance, 80×; b) specific fluorescence of cytoplasm of epithelium of principal glands, 480×.

freeze-dried plasma and a mixture of extracts of definitive human tissues (liver, kidney, spleen, brain, heart, lung), insolubilized with glutaraldehyde [9].

The location of PBG in the tissues of the prostate was determined in freshly frozen sections 10 μ thick by the indirect immunofluorescence method [2], using monospecific antiserum against PBG and luminescent donkey antiserum against rabbit globulin, produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology.

Several available methods of investigation were used to study individual immunochemical and physicochemical properties of PBG (Table 1).

EXPERIMENTAL RESULTS

Immunochemical methods of investigation constantly revealed an antigen in the beta-zone of the antigenic spectrum of definitive prostate tissue with a relative electrophoretic mobility of 0.36 (Fig. 1), a molecular weight of 31,700 ± 2200, and a diffusion coefficient in agarose gel of $1.06 \cdot 10^{-7}$ $cm^2 \cdot sec^{-1}$. According to the results this antigen is one of a series of thermostable simple proteins with no enzyme activity. It was completely salted out by ammonium sulfate at 50% saturation, partially precipitated in the presence of a 5% solution of TCA, of 0.8 M sulfosalicylic acid, and 0.4 M perchloric acid, but was not precipitated by treatment with 0.4% rivanol solution (Table 1).

Since the antigen being studied was not revealed by the methods of investigation used in the composition of other fetal and definitive human tissues (brain, lung, heart, liver, kidney, gastric and intestinal mucosa, bladder, pancreas, adrenal, testis, uterus, ovary), it must be regarded as an organ-specific prostatic antigen.

TABLE 1. Physicochemical Properties of PBG

Physicochemical properties	Result	Method of investigation
Relative electrophoretic mobility in agar gel	0,36±0,005	[4]
Diffusion coefficient in agarose gel, $cm^2 \cdot sec^{-1}$	$1{,}06 \cdot 10^{-7}$	[6]
Mol. wt.	31 700±2 200	[3]
Salting out with ammonium sulfate, % saturation		
complete precipitation	50	
range of precipitation	30—50	
Precipitation:		
by 0.4% rivanol solution	Not precipitated	
0.4 M perchloric acid	3/4 of protein precipitated	
5% TCA solution	7/8 of protein precipitated	
0.8 M sulfosalicylic acid	96% of protein precipitated	
Behavior toward temperature	Stable at 65°C for 30 min	
Staining:		
for proteins (Amido black)	Positive	
for lipoproteins (Sudan black)	Negative	
for glycoproteins (PAS reaction)	Negative	
Enzyme activity:		[8]
phosphatase	Absent	
esterase	"	
protease	"	

TABLE 2. PBG Concentration in Normal and Tumor Tissues of the Prostate (M ± m)

Tissue	Number of observations	Percent of cases in which PBG was found	Mean PBG concentration, mg/100 g tissue	P
Definitive prostate gland	24	100	88,8±8,9	—
Adenoma of the prostate	33	100	57,5±11,4	<0,05
Carcinoma of the prostate	38	84	3,5±0,6	<0,001

PBG was absent from prostatic tissues of fetuses, newborn infants, and children up to 14 years of age, and it began to appear in the prostate at puberty.

PBG is one of the secreted proteins, for it was found in a relatively high concentration (96.0 ± 19.2 mg %) in prostatic fluid and sperm. This antigen was not found in blood serum.

Immunofluorescence analysis (Fig. 2) showed that PBG is localized in the cytoplasm of the large secretory cells of the principal glands of the prostate. Intense specific fluorescence was observed in the perinuclear zone of the cytoplasm and in the apical parts of the cells. Specific fluorescence of secretion located in the lumen of the terminal portions of the glands also was observed.

Comparative immunochemical analysis of the PBG content in normal and tumor tissues of the prostate showed that during tumor growth the PBG level in the prostate fails significantly (Table 2). The fall was particularly marked in malignant prostate tissue ($P < 0.001$). Some tissues of malignant prostate tumors were characterized by antigenic simplification with respect to this organ-specific antigen, for no PBG could be detected in them

by the methods used. The group of these PBG-negative tumors accounted for about 16% of the total number of cases observed.

Besides acid phosphatase, one other organ-specific antigen (PBG) was thus constantly detected in definitive, unchanged prostatic tissues. Synthesis of this antigen by cells of the principal glands commences at puberty, and is accompanied by development and considerable proliferation of the glandular tissue of the prostate. PBG is one of the secreted proteins and is constantly present in prostatic fluid and sperm.

During hyperplasia and carcinoma of the prostate the PBG level falls significantly, probably due to a decrease in the volume of normally functioning prostatic glandular tissue. The results suggest that PBG can serve as an immunochemical marker of tissue differentiation and of functional maturity of the prostate.

The immunochemical test for PBG may also find a place in the biopsy diagnosis of malignant transformation of prostatic tissue.

LITERATURE CITED

1. V. M. Barabanov, in: Textbook of Immunology [in Russian], Moscow (1973), p. 251.
2. I. S. Bashkaev, in: Textbook of Immunology [in Russian], Moscow (1973), p. 330.
3. H. Determann, Gel Chromatography: Gel Filtration, Gel Permeation, Molecular Sieves — A Laboratory Handbook, Springer-Verlag (1969).
4. J. Uriel, in: P. Grabar and J. Burtin, Immunoelectrophoretic Analysis [Russian translation], Moscow (1963), p. 70.
5. N. I. Khramkova and G. I. Abelev, Byull. Éksp. Biol. Med., No. 12, 107 (1961).
6. A. C. Allison and J. H. Humphrey, Nature, 183, 1591 (1959).
7. B. K. Choe, E. J. Pontes, et al., Cancer Treatment Rep., 61, 201 (1977).
8. C. W. Moncure, Med. Coll. Va. Quart., 9, 235 (1973).
9. C. W. Moncure, C. L. Johnston, et al., Cancer Chemother. Rep., 59, 105 (1975).

PROPERTIES OF ANTIGEN-SPECIFIC SUPPRESSOR FACTOR OF IMMUNE SPLEEN CELLS

V. M. Pisarev, S. V. Stukalov, and L. A. Pevnitskii

UDC 612.411.017.1

KEY WORDS: suppression of immune response; antigen-specific suppressor factor; properties of suppressor factor.

An important role in the regulation of the immune response is nowadays ascribed to T suppressor cells (T_S), the function of which is mediated by both specific [3, 5, 11, 12] and nonspecific [4, 10, 14] factors. It was shown previously [1, 15] that after immunization of mice with sheep's red blood cells (SRBC), T_S capable of specifically suppressing the immune response of intact syngeneic recipients appeared in the animals' spleen. The supernatant (SN) obtained after ultrasonic destruction of immune spleen cells (ISC) followed by ultracentrifugation suppressed the immune response of intact recipients to SRBC [2].

The object of this investigation was to study some properties of the suppressor factor (factors) of the spleen cells of immune mice.

EXPERIMENTAL METHOD

Experiments were carried out on adult male CBA/Lac and C57BL/6 mice weighing 18-25 g, obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR. ISC were obtained on the 14th day after intraperitoneal injection of SRBC into mice in a dose of $5 \cdot 10^8$.

Laboratory of Immunogenetics, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.)

Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 90, No. 11, pp. 586-588, November, 1980. Original article submitted June 5, 1980.

0007-4888/80/9011-1568$07.50