Biochimica et Biophysica Acta, 553 (1979) 255—261

BBA 78348

HUMAN GLOMERULAR BASEMENT MEMBRANE ANTIGENIC DETERMINANTS IN HUMAN URINE

PER BYGREN [a], JÖRGEN WIESLANDER [a,b] and DICK HEINEGÅRD [b]

[a] *Department of Nephrology, University Hospital, S-221 85 Lund and* [b] *Department of Physiological Chemistry, University of Lund, Box 750, S-220 07 Lund (Sweden)*

(Received June 20th, 1978)
(Revised manuscript received December 5th, 1978)

Key words: Urinary antigen, Antigenic determinant; (Glomerular basement membrane)

Summary

Antisera against particulate human glomerular basement membrane prepared from cadaver kidneys were raised in rabbits. It was shown that both normal individuals and patients with glomerular and tubular diseases excrete in their urine several antigens reactive with these antibodies. One antigen crossreacted immunologically with an antigen from human glomerular basement membrane while several others did not. One of the urinary antigens and the antigen cross-reacting with the basement membrane were separated from the others by ion exchange chromatography and gel filtration, respectively.

The pattern of antigen excretion differed depending on the underlying renal disease but the multitude of different antigens detected complicates the interpretation of the patterns of excretion in different diseases.

Introduction

Normal urine from many species including man contains substances which are immunologically related to glomerular basement membrane [1]. It has also been shown that urinary substances can induce production of antibasement membrane antibodies in animals [1]. The urinary antigens, however, have not been purified and their anatomical origins have not been determined. It is essential to purify and characterize these immunoreactive substances in order to understand the structure and catabolism of the normal glomerular basement membrane and also to gain information on the degradation of basement membrane in kidney disease. It may be that the quantity and composition of the excreted material is of clinical relevance for assessment of disease activity

and the effects of therapy. In this report the detection and isolation of such substances are presented and their origin is discussed.

Materials and Methods

Preparation of basement membrane [2], fragmentation with enzymes, immunization procedures [3], double diffusion in Agarose gel [4], electroimmunoassay [5], crossed immunoelectrophoresis [6] and immunoelectromigration [7] were performed as described elsewhere [3]. Tandem crossed immunoelectrophoresis was performed according to Krøll [8]. Glomerular filtration rates were measured as Cr-EDTA clearances [9]. Renal biopsy specimens were prepared and classified by light microscopy and immunofluorescence microscopy by Dr. Claus Brun and Dr. Sven Larsen, Kommunehospital, Copenhagen, Denmark.

Experimental procedure

Urine was collected for periods of 1 to 3 days from ten healthy individuals and from patients with kidney disease. Sodium azide was added as a preservative. Urines were concentrated in a dialysis apparatus (Amicon Hollow Fiber Dialyzer Concentrator DC 2) and dialyzed extensively against 0.01 M Tris-HCl, pH 7.0. The pH of each sample was checked and it was centrifuged at 40 000 × *g* for 30 min and applied to a Whatman DE-52 ion-exchange column (2.5 × 30 cm) with a peristaltic pump. The column had been equilibrated with 0.01 M Tris-HCl, pH 7.0, and the same buffer was used to wash the column extensively. The effluent was monitored by following the absorbance at 280 nm. When the absorbance had returned to base-line the column was eluted with a continuous NaCl (0—0.5 M) gradient using the same Tris buffer. The peristaltic pump was used to give a flow rate of 30 ml/h. Fractions (18 ml) were collected and screened for antigenic activity by electroimmunoassay. The separate peaks were pooled as indicated in Fig. 1 and concentrated by ultrafiltration (Amicon type UM 2). Four fractions were recovered and will be referred to as DE 0.12; DE 0.15; DE 0.18 and DE 0.22, respectively. Each fraction was then chromatographed on a Sepharose 6B (Pharmacia Fine Chemicals) column (1.8 × 180 cm) equilibrated with 0.15 M Tris-HCl, pH 7.4. Fractions of 8 ml were collected and screened for antigenic activity. Tubes containing antigenic material were pooled and concentrated by ultrafiltration on UM 10 membranes (Amicon Corp.). Fractions referred to as DE 0.12-6B 1; 6B 2 and 6B 3 were obtained. They were tested for immunological crossreactivity against each other and against fractions prepared by treating human glomerular basement membrane with collagenase, pepsin and trypsin.

Results and Discussion

Human glomerular basement membrane antigens in normal urine

Electroimmunoassay and the fused rocket technique were used to demonstrate that several substances in human urine react with rabbit antibodies against particulate human glomerular basement membrane. These antigenic substances were incompletely or not at all separated by Sepharose 6B

chromatography (unpublished). Ion-exchange chromatography on DEAE-cellulose (DE 52), on the other hand, was used to separate partly the antigenic fragments (Fig. 1). No antigen was eluted with 0.01 M Tris, pH 7.0, from the column. The ion-exchange column was then eluted with a gradient of NaCl. Antigenic determinants were eluted at concentrations of about 0.12 M, 0.15 M, 0.18 M and 0.22 M NaCl, respectively. These fractions were pooled as indicated in Fig. 1 and will be referred to as DE 0.12 to DE 0.22, respectively. Tandem crossed immunoelectrophoresis and immunoelectromigration were used to show that the fraction designated DE 0.22 had a major antigenic determinant which crossreacted with a fraction solubilized from basement membrane by collagenase digestion (Fig. 2). Further fractionation on Sepharose 6B indicated that the antigen found in the urine was very polydisperse and/or heterogeneous. Electroimmunoassay against antiglomerular basement membrane antibodies of the material from the Sepharose 6B fractions showed that antigens were present in most of the fractions containing protein. The effluent was pooled as indicated in Fig. 3. The antigens in the three fractions pooled were shown to be immunologically identical by using immunoelectromigration.

The material eluted first from the DE 52 column, DE 0.12, was further fractionated on Sepharose 6B. Again protein and antigenic material was not separated. Fused rocket analysis of the Sepharose 6B fractions indicated the presence of 4 overlapping antigenic determinants (Fig. 4). Neither the Sepharose 6B fractions nor the original DE 0.12 fraction showed any immunological identity with DE 0.22 nor with material solubilized from basement membrane by collagenase, trypsin or pepsin digestion. The fractions eluted from the DE 52 column at intermediate ionic strength contained mixtures of antigenic determinants which were not separated into distinct entities by ion-exchange chromatography or by gel filtration. It appears then, that urine contains at least two fractions with different antigenic determinants, one (DE 0.22) cross-reacting immunologically with glomerular basement membrane fragments. The other fraction (DE 0.12) reacted with anti-basement membrane antibodies but not with fragments liberated from glomerular basement membrane by proteolytic digestion. It is possible that some of the membrane determinants are destroyed by the action of the proteases or alternatively not solubilized.

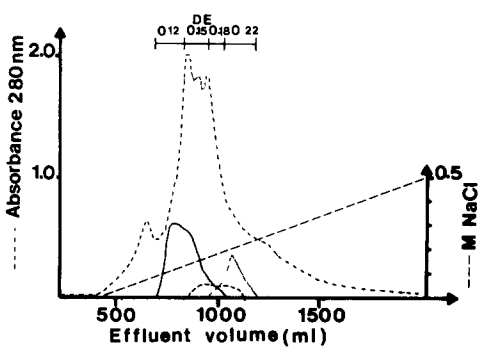

Fig. 1. Elution profile of normal urine on ion exchange chromatography. Inserted traces represent the result of a fused rocket analysis of pooled fractions (indicated at the top of the diagram) against anti-human glomerular basement membrane gamma globulin.

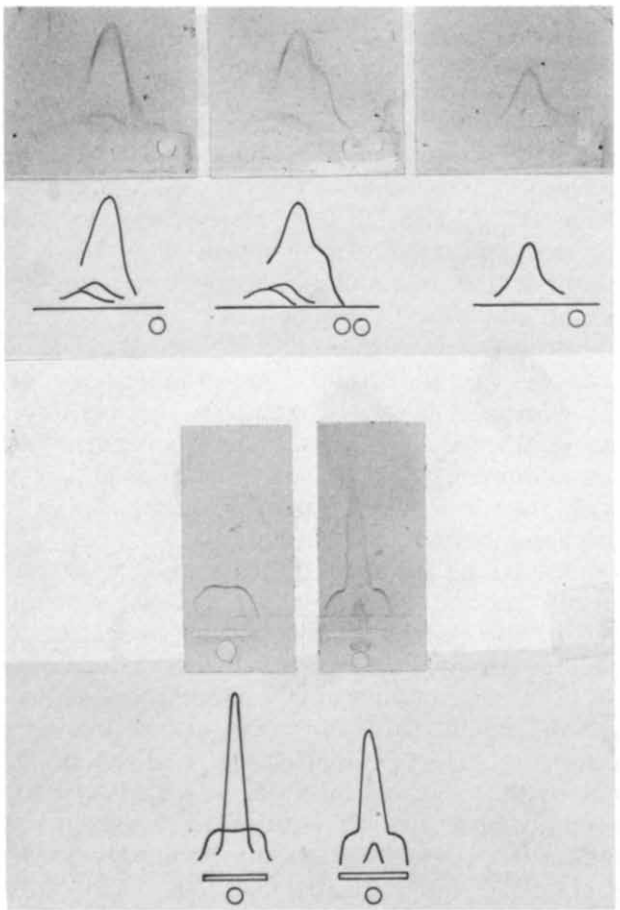

Fig. 2. Upper left: Crossed immunoelectrophoresis of DE 0.22 pool from human urine. Upper right: Crossed immunoelectrophoresis of Sepharose 6B fraction where the crossreacting human glomerular basement membrane antigen is isolated [4]. (Collagenase digested basement membrane fraction 48.) Upper center: Tandem crossed immunoelectrophoresis analysis of the two fractions, showing the crossreaction. Lower left: Immunoelectromigration with collagenase digested basement membrane fraction 48 h in the slit and urine fraction DE 0.12 in the well. Absence of crossreactivity shown. Lower right: Immunoelectromigration with collagenase digested basement membrane fraction 48 h in the slit and urine fraction DE 0.22 in the well. Total identity shown.

As discussed, the fractionation procedures used are ineffective in removing non-related proteins and can therefore not be used for purification. They can, however, be useful tools to fractionate and separate molecules with different antigenic determinants.

Antigens in pathological human urine

Experiments were carried out to compare the patterns and quantities of glomerular basement membrane related antigenic fragments in urine from patients with kidney disease with the patterns obtained with samples of normal human urine. The clinical data and the diagnoses of the patients studied are summarized in Table I. When samples prepared from the two patients recovering from acute tubular insufficiency were tested by immunological methods against

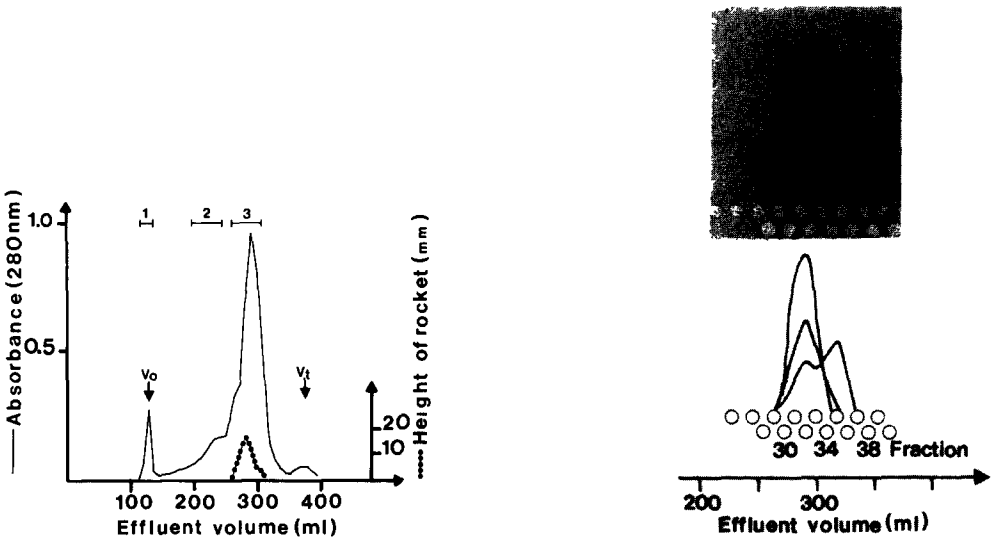

Fig. 3. Elution profile of Sepharose 6B chromatography of a DE 0.22 fraction pool from normal urine. Also shown: The rocket analysis of antigens against anti-human glomerular basement membrane gamma-globulin. Fractions were pooled as indicated at the top.

Fig. 4. Fused rocket analysis of antigens in the Sepharose 6B eluate of DE 0.12 pool from human urine. Anti-human glomerular basement membrane gamma-globulin in the gel. Sepharose 6B fractions in wells as indicated. One continuous precipitation line represents one antigen-antibody system.

those obtained from normal urines it was found that the corresponding fractions from the DE 52 column contained crossreacting antigens. The urines from several patients with chronic glomerulonephritis showed more complex immunological reactivity. The first fraction from the DE 52 column (DE 0.12) in all but one case showed non-identity with the corresponding fraction in normal urine. The last eluted fraction (DE 0.22) showed an even more complex picture.

TABLE I

CLINICAL AND LABORATORY DATA ON PATIENTS

Patient	Morphological findings	Glomerular deposits (Immunofluorescence)	Clinical diagnosis	Glomerular filtration rate (ml/min)
1	Epimembraneous gln *	IgG, C3 granular	Nephrotic syndrome	40
2	Epimembraneous gln	IgG, C3 granular	Nephrotic syndrome	50
3	Diffuse proliferative gln	IgG, C3 granular	Nephrotic syndrome	23
4	Extracapillary gln	Non-linear fibrin	Acute gln (non-streptococcal)	creatinine in serum 475 μmol/l
5	Tubular cell and interstitial changes	None	Acute tubular insufficiency	24
6	Tubular and interstitial changes	None	Acute tubular insufficiency	20

* gln, glomerulonephritis.

TABLE II

TRYPSIN, COLLAGENASE AND PEPSIN SOLUBILIZED HUMAN GLOMERULAR BASEMENT MEMBRANE AND ITS IDENTITIES WITH DIFFERENT URINES

HGBM, human glomerular basement membrane.

		Pepsin HGBM	Trypsin HGBM	Collagenase HGBM
Glomerulonephritic urine				
Patient I	DE 0.12	—	—	—
	DE 0.22	—	+	+
Patient II	DE 0.12	—	—	—
	DE 0.22	—	+	+
Patient III	DE 0.12	—	—	—
	DE 0.22	—	+	—
Patient IV	DE 0.12	—	—	—
	DE 0.22	—	+	+
Tubular insufficiency urine				
Patient V	DE 0.12	—	—	—
	DE 0.22	—	—	+
Patient VI	DE 0.12	—	—	—
	DE 0.22	—	+	+
Normal urine				
	DE 0.12	—	—	—
	DE 0.22	—	+	+

Both crossreactivity with the corresponding fractions from normal urine, as well as non-identity was observed. It is noteworthy, however, that all urines contained antigenic materials eluting at the same salt concentration and reacting with the antibodies against glomerular basement membrane regardless of crossreactivity between normal and pathological urine or glomerular basement membrane fragments prepared by protease digestion. It can be concluded that substances with different antigenic specificities but still related immunologically to basement membrane are probably also excreted in kidney disease. It may be that the pattern of antigenic determinants excreted may depend on the underlying renal disease.

Antigens in glomerular basement membrane crossreacting with urinary antigens

Three samples of human glomerular basement membrane were digested with pepsin, trypsin and collagenase and tested for crossreactivity with fractions from normal and pathological human urines. The results are summerized in Table II. Pepsin-treated membrane gave no clear identity with any of the urine antigens. The digests of basement membrane with trypsin and with collagenase, respectively, crossreacted only with the urinary fraction eluted with 0.22 M NaCl from the DE 52 column.

General Discussion

It is possible that most of the urinary antigens do not reflect gomerular basement membrane constituents. The particulate basement membrane preparations used for immunization contained several contaminants, primarily plasma constituents and cellular material. The sera obtained from the immunized

rabbits reacted with many, if not all, plasma proteins. Therefore the antisera were adsorbed with pooled human plasma to remove this reactivity. Antisera did not react when tested against red cell membrane preparations, thrombocytes and the Tamm-Horsfall glycoprotein isolated from urine. It is likely, then, that the purified antiserum used in this investigation does not react with cellular material and therefore that it is specific for basement membrane fragments.

The anatomical origin of the urinary substances was further investigated. As discussed above at least one antigen found in the urine crossreacted with the soluble fraction of collagenase digested basement membrane. On the other hand antigens reacting with the antisera but not showing reaction of identity with any of the urinary antigens were found in extracts of non-renal tissues such as aorta, lens capsule and placenta. It is possible that these antigens represent the basement membrane present in all the tissues tested. In support of this, fractions derived from tissues devoid of vessels and basement membranes, such as cartilage or red cell membranes and platelets, did not show any reaction with anti-glomerular basement membrane antiserum. The crossreacting antigens of non-renal origin are, however, too large to pass the normal glomerular filter. Therefore, the crossreacting antigens excreted in normal urine are probably derived from sites distal to the filtration barrier. The excretion of these antigens then, can probably be used an an indicator of the normal basement membrane turnover. Efforts are now under way to purify the antigens in order to correlate their structure more closely to that of human glomerular basement membrane antigens and also to antibody formation against basement membrane in human disease.

Acknowledgements

Financial support was provided by the Medical Faculty, University of Lund, Segerfalks stiftelse and AB Gambro (fund), Lund.

References

1 Mc Phaul, J.J. and Dixon, F.J. (1969) J. Exp. Med. 130, 1395—1409
2 Westberg, N.G. and Michael, A.F. (1970) Biochemistry 9, 3837—3846
3 Wieslander, J., Bygren, P. and Heinegård, D. (1979) Biochim. Biophys. Acta 553, 244—254
4 Ouchterlony, O. (1962) Prog. Allergy 6, 30—154
5 Laurell, C.B. (1966) Anal. Biochem. 15, 45—52
6 Clarke, H.G.H. and Freemen, T. (1967) in Protides of Biological Fluids (Peeters, H., ed.), Vol. 14, pp. 503—509, Pergamon Press, Oxford
7 Grubb, A. (1972) Scand. J. Clin. Lab. Invest. 124, Suppl. 59—65
8 Krøll, I. (1973) Scand. J. of Imm. 2, Suppl. 1, 57—59
9 Broechner-Mortensen, I. and Rodbro, P. (1976) Scand. J. Clin. Lab. Invest. 36, 35—37