Glomerular Basement Membrane Proteoglycans Are Derived from a Large Precursor

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Abstract. The basement membrane heparan sulfate proteoglycan produced by the Englebreth-Holm-Swarm (EHS) tumor and by glomeruli were compared by immunological methods. Antibodies to the EHS proteoglycan immunoprecipitated a single precursor protein (M_r = 400,000) from [^{35}S]methionine-pulsed glomeruli, the same size produced by EHS cells. These antibodies detected both heparan sulfate proteoglycans and glycoproteins in extracts of unlabeled glomeruli and glomerular basement membrane. The proteoglycans contained core proteins of varying size (M_r = 150,000 to 400,000) with a M_r = 250,000 species being predominant. The glycoproteins are fragments of the core protein which lack heparan sulfate side chains. Antibodies to glomerular basement membrane proteoglycan immunoprecipitated the precursor protein (M_r = 400,000) synthesized by EHS cells and also reacted with most of the proteolytic fragments of the EHS proteoglycan. This antibody did not, however, react with the P44 fragment, a peptide situated at one end of the EHS proteoglycan core protein. These data suggest that the glomerular basement membrane proteoglycan is synthesized from a large precursor protein which undergoes specific proteolytic processing.

CERTAIN tumor cells such as the Englebreth-Holm-Swarm (EHS)[1] tumor, parietal yolk sac carcinomas, embryonal carcinoma-derived endodermal cells, and NMuMG epithelial cells, have been useful model systems for characterizing components of basement membranes. These components include laminin (Timpl et al., 1979; Chung et al., 1979; Martinez-Hernandez et al., 1982; Wewer, 1984), collagen IV (Orkin et al., 1977; Timpl et al., 1978; Kleinman et al., 1982), entactin/nidogen (Carlin et al., 1981; Timpl et al., 1983), heparan sulfate proteoglycan (Hassell et al., 1980; Oohira et al., 1982; Tyree et al., 1984; Ledbetter et al., 1985), and a proteoglycan containing both heparan sulfate and chondroitin sulfate side chains (Jalkanen et al., 1985; Kato et al., 1987). In addition, cDNA clones to collagen IV (Schwartz et al., 1986), laminin (Sasaki et al., 1987) and entactin (Durkin et al., 1987) have been obtained using RNA prepared from tumors. These and other studies have shown that native basement membranes contain components similar, at least at the immunological level, to those in tumors (Laurie et al., 1982; Martinez-Hernandez and Armmenta, 1983; Timpl and Dziadek, 1986; Martin and Timpl, 1987).

Heparan sulfate proteoglycans isolated to date from native basement membranes are, however, considerably different from those isolated from basement membrane producing tumors (for reviews see Hassell et al., 1986; Gallagher et al., 1986; Poole, 1986). The major EHS proteoglycan (M_r ~600,000) has a large (M_r = 400,000) core protein with three heparan sulfate side chains of M_r = 65,000 (Hassell et al., 1985*b*; Kato et al., 1985; Paulsson et al., 1987). Proteolytic fragmentation indicates that the core protein consists of two major domains; a trypsin sensitive domain (M_r = 200,000) containing the heparan sulfate side chains and a trypsin resistant domain (M_r = 200,000) from which two small peptides can be generated by V-8 protease (M_r = 44,000 and 46,000) that are immunologically distinct from one another (Ledbetter et al., 1987). The glomerular proteoglycan, (Kanwar et al., 1981; Stow et al., 1983; Parthasarathy and Spiro, 1984; Klein et al., 1986) in contrast, is smaller (M_r = 130,000–200,000) with four heparan sulfate side chains of M_r = 12,000–30,000 attached to a core protein ranging from M_r = 18,000 (Kanwar et al., 1984) to M_r = 143,000 (Edge and Spiro, 1987). It is possible that the variability in core protein size may be due to proteolysis. This has been noted in the EHS tumor, where a portion of the population of the proteoglycan was found to be proteolytically processed to produce smaller proteoglycans with M_r

1. *Abbreviation used in this paper*: EHS, Englebreth-Holm-Swarm.

= 95,000–130,000 core proteins (Ledbetter et al., 1985; Hassell et al., 1985*b*).

Despite the size differences between the EHS and glomerular proteoglycans, antibodies to the EHS proteoglycan react strongly with glomerular basement membranes as well as with all other basement membranes examined (Hassell et al., 1980; Laurie et al., 1982; Mynderse et al., 1983). In this study we have used antibodies to the EHS proteoglycan to characterize the immunoreactive material present in the glomerular basement membrane and have studied the reaction of glomerular proteoglycan antibodies with the EHS proteoglycan. The results of this study indicate that the EHS and glomerular proteoglycan are similar but that the glomerular proteoglycan is proteolytically processed to produce smaller proteoglycans.

Materials and Methods

Tissue and Cell Preparation

Glomeruli were isolated from kidney cortices removed from 175 g male Sprague–Dawley rats by serial sieving through wire meshes. The glomeruli were retained on 150 and 200 mesh screens and collected by low speed centrifugation (Brown et al., 1981). These preparations contained less than 5% tubular elements and the cells excluded trypan blue both before and after in vitro radiolabeling. Glomerular basement membrane was prepared from glomeruli by sequential treatment with distilled water, DNAse, and deoxycholate as previously described (Brown et al., 1981).

The EHS tumor was maintained by serial passage in the hind limbs of C57BL mice and harvested as previously described (Orkin et al., 1977). Cells were isolated from freshly harvested EHS tumor by using a combination of mechanical disruption and dispase digestion (Ledbetter et al., 1985). The isolated cells were plated at 4×10^6 cells/35-mm dish and were cultured overnight in NCTC 109 containing 10% fetal bovine serum.

EHS cells in culture were radiolabeled for 20 min with 500 μCi of [^{35}S]methionine/ml in medium free of serum and methionine (Ledbetter et al., 1985). The cells were scraped from the dish at the end of the labeling period, pelleted by centrifugation, and were stored at −70°C. Isolated glomeruli were radiolabeled in organ culture using the same procedure, but with low speed centrifugation (400 *g* for 3 min) to pellet the glomeruli and to permit media changes. Approximately 100 μl of pelleted glomeruli were suspended and radiolabeled in 1 ml of media containing the isotope. The radiolabeled glomeruli were collected by centrifugation and stored at −70°C.

Unlabeled glomeruli and glomerular basement membranes were either briefly sonicated in 2% SDS containing 0.01 M phosphate buffer, pH 7.4, and heated at 100°C for 3 min or extracted in 4 M guanidine–HCl containing 0.05 M sodium acetate, 0.01 M EDTA, 0.005 M benzamidine-HCl, and 0.1 M 6 aminohexanoic acid, pH 6.5, overnight at 4°C on a rocker. The protein content in the SDS extract was measured with Folin reagent (Lowry et al., 1951). The guanidine extract was dialyzed against 6 M urea containing 0.15 M NaCl, 0.1% CHAPS and 0.05 M Tris–HCl, pH 6.8, and applied to a column (1.5 × 1.5 cm) of DEAE Sephacel equilibrated with the same solvent. The eluent was monitored at 280 nM. After the unbound glycoprotein fraction was washed from the column the bound proteoglycan fraction was eluted with the same solvent containing 1.0 M NaCl. The glycoprotein and proteoglycan fractions were dialyzed against distilled water, lyophilized, and reconstituted in distilled water. An aliquot of the proteoglycan fraction was digested with heparitinase (Hassell, et al., 1985*b*; Kato et al., 1985). The completeness of hepartinase digestion was checked by the addition of more hepartinase. The samples were adjusted to 2% SDS and 0.01 M sodium phosphate buffer, pH 7.4, and boiled for 3 min before PAGE. Molecular weight markers, obtained from Bio-Rad Laboratories (Richmond, CA), and laminin were used as standards.

Antigen Purification and Immunologic Procedures

The EHS proteoglycan was purified from urea extracts of the EHS tumor (Ledbetter et al., 1987). After washing the tissue with 3.4 M NaCl, the residue is extracted with 6 M urea and the large, low density proteoglycan was isolated by sequential use of DEAE Sephacel column chromatography, CsCl density gradient centrifugation, and Sepharose CL-4B column chromatography. Proteolytic digestion of this purified proteoglycan was conducted in 0.02 M Tris-HCl, pH 8.0, containing 1 mg/ml of proteoglycan and 10 μg/ml of either V8 protease (Miles Laboratories, Inc., Naperville, IL) or Trypsin (Sigma Chemical Co., St. Louis, MO) at 37°C (Ledbetter et al., 1987). Purification of the P200 P46 and P44 core protein fragments was accomplished by sequential chromatography on sepharose CL-6B and DEAE 5PW (Ledbetter et al., 1987). Glomeruli and glomerular basement membrane were isolated from fresh bovine kidneys as previously described (Parthasarathy and Spiro, 1984). The glomerular proteoglycan was purified from preparations of bovine glomerular basement membrane by extraction with 4 M guanidine–HCl followed by dialysis against 7 M urea and chromatography on a DEAE Sephacel column (Brenchley, P. E., M. A. J. Dickinson, and J. C. Anderson, manuscript in preparation). Antibodies to the EHS proteoglycans, the glomerular proteoglycan and laminin were raised in rabbits using the same method (Hassell et al., 1980; Hassell et al., 1985*b*; Brenchley, P. E., M. A. J. Dickinson, and J. C. Anderson, manuscript in preparation; Foidart et al., 1980).

Antibodies to the EHS proteoglycan were purified by affinity chromatography. EHS proteoglycan core protein was produced by heparitinase digestion of 10 mg of proteoglycan and isolated by chromatography on Sepharose CL-4B in 4 M guanidine–HCl. The purified core protein was coupled to 20 ml of CNBr-activated Sepharose CL-4B according to the manufacturer's (Pharmacia Fine Chemicals, Piscataway, NJ) instructions. Antibodies were purified from antisera by circulating 10 ml of antiserum overnight at 4°C, removing unbound material with PBS and bound IgG with 4M guanidine–HCl, 0.05 M Tris–HCl, pH 7.0. The eluted IgG was dialyzed against 0.15 M NaCl, 0.01 M sodium phosphate, pH 7.4, and then adjusted to the same volume of serum originally applied to the coupled resin.

Immunoprecipitation of protein from extracts of radiolabeled cells and glomeruli was accomplished as described previously (Ledbetter et al., 1985). In brief, cells were lysed in a buffered detergent solution and a portion of the lysate was mixed with Protein A–Sepharose beads pre-bound with the antibodies or antisera. The beads were then washed and the antigen–antibody complex on the beads was solubilized in 0.01 M sodium phosphate buffer, pH 7.4, containing 2% SDS and 0.1 M dithiothreitol by heating 100°C for 3 min. The solubilized proteins were separated by PAGE in 0.1% SDS (Laemmli, 1970) and gels were prepared for fluorography with Autofluor (National Diagnostics, Somerville, NJ) according to the manufacturer's instructions.

Electrophoretic transfer of proteins to nitrocellulose from polyacrylamide gels was accomplished in a buffer containing 20% methanol at 90 V overnight at 4°C (Towbin et al., 1979). Subsequent steps were done at room temperature. Unused binding sites on the nitrocellulose were blocked with 3% gelatin in TBS (0.02 M Tris HCl, pH 7.5, containing 0.5 M NaCl), exposed to the anti-proteoglycan antibody or antisera diluted 1:200 in 1% gelatin in TBS for 2 h, rinsed in TBS, exposed to horseradish-peroxidase-conjugated goat anti-rabbit IgG diluted 1:2,000 in 1% gelatin in TBS for 2 h, rinsed again in TBS, and the color developed with 0.05% 4-chloro-1-naphthol in TBS containing 15% methanol and 0.001% H_2O_2. Unblocked nitrocellulose filters were stained for protein with amido black. Enzyme-linked immunoabsorbent assays (ELISA) were conducted, as previously described (Rennard et al., 1981), using polystyrene plates, Voller's buffer for coating fragments on the plates, PBS-tween as the rinsing buffer, and the second antibody described above.

Results

The immunostaining of kidney sections with antibodies to the EHS proteoglycan indicated that the glomerular basement membrane contains immunologically related material (Hassell et al., 1980; Laurie et al., 1982; Mynderse et al., 1983). Consequently, to identify this material, extracts of both glomeruli and glomerular basement membrane were subjected to electrophoresis and examined for reaction with the antibodies to the EHS proteoglycan by Western blot analysis. Extracts of glomeruli contained three diffuse broad bands (M_r = 400,000, 200,000, and 150,000) which reacted with the antibody to the EHS proteoglycan (Fig. 1, lane *A*). Extracts of glomerular basement membrane contained the M_r = 200,000 and 150,000 bands as well as several smaller components that reacted with the antibody (Fig. 1, lane *B*).

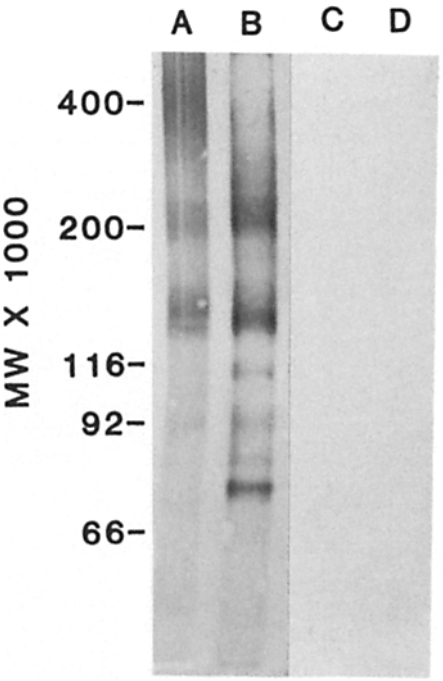

Figure 1. Immunoreaction of antibodies to EHS proteoglycan and preimmune serum with SDS extracts of glomeruli and glomerular basement membrane subjected to electrophoresis on polyacrylamide gels and transferred to nitrocellulose. Extracts from glomeruli containing 80 μg protein (lanes *A* and *C*) and extracts from glomerular basement membrane containing 4 μg protein (lanes *B* and *D*) were subjected to electrophoresis without reduction on polyacrylamide gels containing 5% running gels. The material transferred to nitrocellulose was reacted with antibodies to EHS proteoglycan (lanes *A* and *B*) or preimmune serum (lanes *C* and *D*).

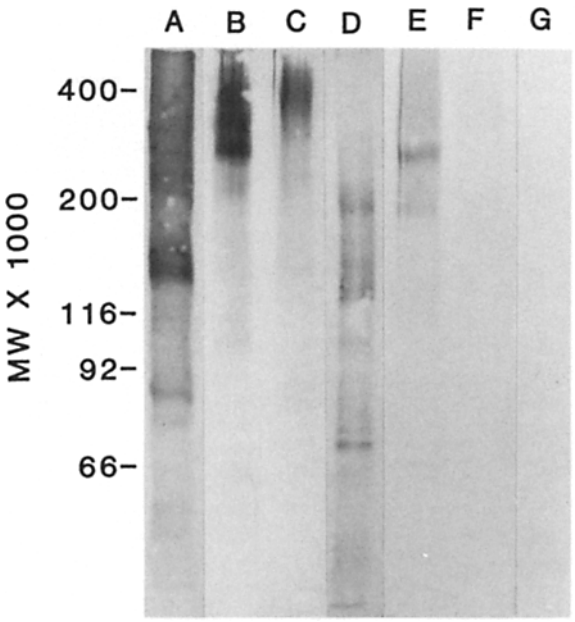

Figure 2. Immunoreaction of antibodies to EHS proteoglycan with glycoprotein and proteoglycan fractions from extracts of glomeruli and glomerular basement membranes subjected to electrophoresis on polyacrylamide gels and transferred to nitrocellulose. Glycoprotein and proteoglycan fractions were isolated from guanidine–HCl extracts by column chromatography on DEAE-Sepharose and applied unreduced to 5% polyacrylamide gels (Lane *A*) Glycoprotein fraction from glomeruli; (lane *B*) proteoglycan fraction from glomeruli digested with heparitinase; (lane *C*) proteoglycan fraction from glomeruli; (lane *D*) glycoprotein fraction from glomerular basement membranes; (lane *E*) proteoglycan fraction from glomerular basement membranes digested with heparitinase; (lane *F*) proteoglycan fraction from glomerular basement membranes; (lane *G*) heparitinase. Equivalent amounts of immunoreactive material was loaded in lanes *A*, *B*, and *C*, and in lanes *D*, *E*, and *F*.

No reaction was observed with preimmune serum (Fig. 1, lanes *C* and *D*). It should be noted that the amount of protein subjected to electrophoresis from the two extracts was adjusted (80 μg for glomeruli; 4 μg for glomerular basement membrane) to produce approximately equivalent immunostaining on the nitrocellulose (Fig. 1, lanes *A* and *B*). The 20-fold higher immunoreactivity of the glomerular basement membrane protein over the glomeruli protein reflects an enrichment due to purification of basement membrane and indicates the antigenic determinants detected in the extracts of glomeruli were primarily from the basement membrane and not from the cells.

The glycoproteins and proteoglycans in guanidine extracts of glomeruli and glomerular basement membranes were separated by DEAE-Sephacel column chromatography, subjected to electrophoresis, and then analyzed for reactivity with the antibody to the EHS proteoglycan in Western blots. The pattern of immunoreactive bands in the glycoprotein fractions from both the glomeruli (Fig. 2, lane *A*) and the glomerular basement membrane (Fig. 2, lane *D*) was similar to that previously observed in unfractionated extracts of these tissues (Fig. 1, lanes *A* and *B*, respectively). The M_r = 400,000 band in the unfractionated extract of glomeruli (Fig. 1, lane *A*), however, appeared reduced or absent in the glycoprotein fraction from glomeruli (Fig. 2, lane *A*) and the undigested proteoglycan fraction from glomeruli contained a broad immunoreactive band at M_r = 400,000 (Fig. 2, lane *C*). Heparitinase digestion of either glycoprotein fraction did not alter the migration position of the immunoreactive material (not shown). In contrast, Heparitinase digestion of the proteoglycan fraction from glomeruli produced a major immunoreactive band at M_r = 250,000 (Fig. 2, lane *B*) and somewhat lesser amounts of two broad but larger bands (M_r = 300,000 and 400,000). These bands are core proteins from the glomerular proteoglycan that are immunologically related to the EHS proteoglycan. Heparitinase digestion of the proteoglycan fraction from the glomerular basement membrane produced immunoreactive core proteins of M_r = 250,000 and 200,000 as well as disperse, faint core protein at M_r = 150,000 (Fig. 2, lane *E*). These core proteins were not detected either in the undigested proteoglycan fractions (Fig. 2, lane *F*) or in the heparitinase preparation (Fig. 2, lane *G*). It was necessary to use four to five times more of the heparitinase digested proteoglycan fraction than the glycoprotein fraction to produce the intensity of staining observed in these blots. This could be due to poor transfer of core proteins to nitrocellulose. Alternatively, it would indicate that the majority of the immunological determinants detected in the glomerular basement membrane lack glycosaminoglycan side chains in situ. The reduction or absence

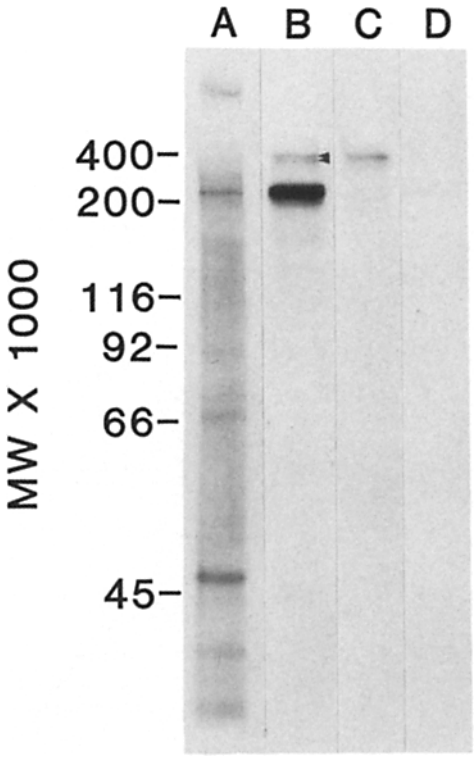

Figure 3. Fluorograph of [^{35}S]methionine-labeled proteins immunoprecipitated from a lysate of pulse-labeled glomeruli and were subjected to electrophoresis on polyacrylamide gels. The lysate was run directly on the gels (lane *A*) or proteins in the lysate were immunoprecipitated with antibodies to laminin (lane *B*), antibodies to the EHS proteoglycan (lane *C*) and preimmune serum (lane *D*). Samples were reduced before electrophoresis. The running gel was 7.5% polyacrylamide. Arrowheads denote the position of the doublet in lane *B*.

of staining in lanes containing the same amount of undigested proteoglycan (Fig. 2, lanes *C* and *F*) may be due to the limited migration of the intact proteoglycan into the polyacrylamide gel, its failure to transfer well to the nitrocellulose paper, reduced binding of proteoglycan to nitrocellulose or steric hinderance (by heparan sulfate chains) of antibody binding.

Next, we metabolically radiolabeled glomeruli in vitro for 20 min with [^{35}S]methionine and used antibodies to the EHS proteoglycan to identify the precursor protein(s) to these immunoreactive proteins and proteoglycans. The material synthesized in only 20 min would still be inside the cells and would not have all posttranslational modifications, such as glycosaminoglycan side chain addition or proteolytic processing (Ledbetter et al., 1985). Electrophoresis of the glomerular lysate on SDS–polyacrylamide gels followed by fluorography showed a variety of labeled proteins (Fig. 3, lane *A*). Antiserum to laminin was used as a positive control and to produce high molecular weight markers. This antiserum immunoprecipitated a major band at M_r = 220,000 which represents the B chain(s) of laminin, and a doublet at M_r = 400,000 (Fig. 3, lane *B*). The lower band in the doublet is likely to be the A chain of laminin while the upper band is probably the precursor protein to the proteoglycan which has been shown to coprecipitate with antibodies to laminin (Ledbetter et al., 1985). Indeed, antibodies to the EHS proteoglycan immunoprecipitated this precursor protein (M_r = 400,000) and no other proteins from the lysate (Fig. 3, lane *C*). This protein can be observed in the whole lysate but only in overexposed fluorograms (not shown). Preimmune serum did not immunoprecipitate any proteins from the lysate (Fig. 3, lane *D*). These results indicate that the cells of glomeruli in organ culture synthesize the same size chains of laminin as well as the same size (M_r = 400,000) precursor protein to the EHS proteoglycan as was previously identified by immunoprecipitation from lysates of labeled EHS cells (Ledbetter et al., 1985).

The ability of antibodies to the glomerular proteoglycan to immunoprecipitate the M_r = 400,000 precursor protein synthesized by EHS cells was also examined. Lysates of EHS cells, radiolabeled in culture for 20 min with [^{35}S]methionine, were immunoprecipitated with antibodies to laminin (Fig. 4, lane *A*), to the EHS proteoglycan (Fig. 4, lane *B*), and to the glomerular proteoglycan (Fig. 4, lane *C*). Antibodies to the glomerular proteoglycan (Fig. 4, lane *C*) immunoprecipitated the same precursor protein (M_r = 400,000) identified by the antibodies to the EHS proteoglycan (Fig. 4, lane *B*). This indicates that EHS and glomerular proteoglycans share some common antigenic determinants. The antibodies to the glomerular proteoglycan also immunoprecipitated a small amount of the A and B chains of laminin. The lower molecular weight components observed in lanes *B* and *C* (Fig. 4) were present in precipitates obtained with preimmune serum (Fig. 4, lane *D*) and are considered to be nonspecific.

Trypsin and V-8 protease digests of the EHS proteoglycan were subjected to electrophoresis and then reacted with antibody to the glomerular proteoglycan in Western blots. Trypsin produced a major fragment of M_r = 200,000 (P200) (Fig. 5, lane *A*) whereas V-8 protease produces a number of

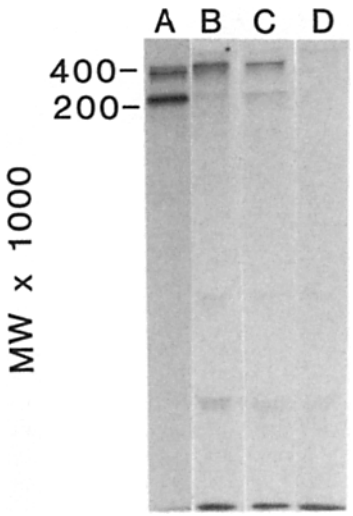

Figure 4. Fluorograph of [^{35}S]methionine-labeled proteins immunoprecipitated from a lysate of pulse labeled EHS cells and were subjected to electrophoresis on polyacrylamide gels. Immunoprecipitates from antibodies to laminin (lane *A*), antibodies to EHS proteoglycan (lane *B*), antibodies to glomerular proteoglycan (lane *C*) and preimmune serum (lane *D*). Samples were reduced before electrophoresis. Running gel was 7.5% polyacrylamide.

fragments (Fig. 5, lane *D*) and those at M_r = 46,000 (P46) and 44,000 (P44) are derived from the P200 fragment (Ledbetter et al., 1986). The trypsin (Fig. 5, lanes *B* and *C*) and the V-8 protease (Fig. 5, lanes *E* and *F*) produced peptides reacted similarly with antibodies to both the glomerular and the EHS proteoglycan. This extensive cross-reaction suggests that the EHS and glomerular proteoglycans share many antigenic determinants. The antibodies to these two proteoglycans did not, however, react equally with all the peptide fragments. For example, antibodies to the glomerular proteoglycan did not appear to react with the P44 and P46 fragments (Fig. 5, lane *F*) with the same relative intensity as the antibody to the EHS proteoglycan (Fig. 5, lane *E*). Consequently, we used the more quantitative ELISA on microtiter plates to measure the reactivity of these antibodies with purified P44, P46, and P200 fragments from the EHS proteoglycan. Antibodies to the EHS proteoglycan reacted strongly with the P44 and P46 fragments and even more strongly with the P200 fragment (Fig. 6 B) as previously shown (Ledbetter et al., 1986). In comparison, antibodies to the glomerular proteoglycan reacted strongly with the P46 and P200 fragment but reacted only very weakly with the P44 fragment (Fig. 6 *A*). These results indicate that while immunological determinants on the P46 peptide were present on the glomerular proteoglycan used as antigen, the determinants on the P44 peptide were substantially reduced or absent from the glomerular poteoglycan.

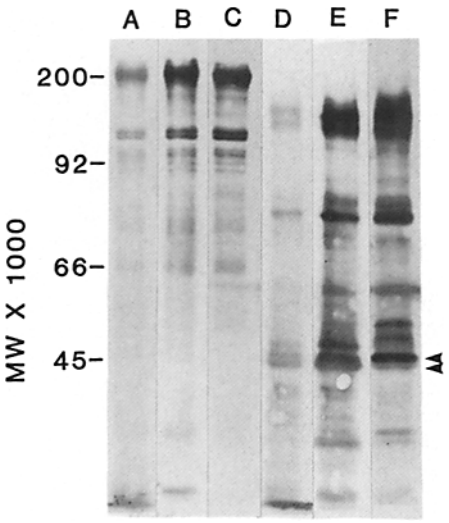

Figure 5. Immunoreaction of antibodies to basement membrane proteoglycans with proteolytic digests of EHS proteoglycan were subjected to electrophoresis on polyacrylamide gels and transferred to nitrocellulose. Proteoglycan was digested with either trypsin for 2 h or V8 protease for 8 h and applied without reduction to polyacrylamide gels containing 5% running gels. 10 μg of digested proteoglycan was applied for amido black staining and 3 μg for immunostaining. Lane *A*, amido black staining of trypsin digests; lane *B* immunostaining of trypsin digests with antibodies to EHS proteoglycan; lane *C*, immunostaining of trypsin digests with antibodies to glomerular proteoglycan; lane *D*, amido black staining of V8 protease digests; lane *E*, immunostaining of V8 digests with antibodies to EHS proteoglycan; lane *F*, immunostaining of V8 digests with antibodies to glomerular proteoglycan. Arrowheads point to migration position of M_r = 44,000 and 46,000 fragments produced by digestion with V8 protease.

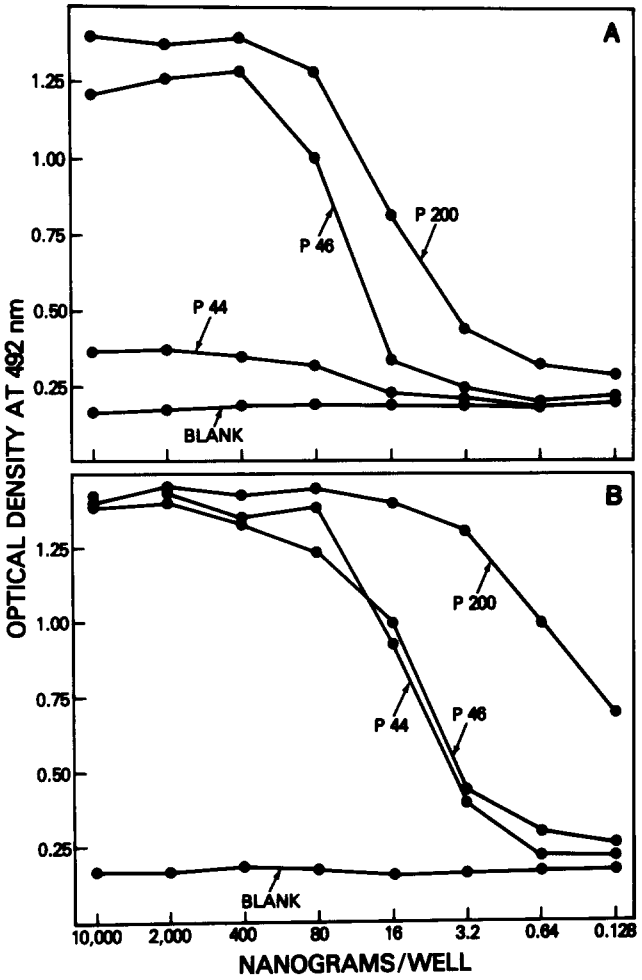

Figure 6. Immunoreaction of antibodies to basement membrane proteoglycans with the P44, P46 and P200 fragments from the EHS proteoglycan using ELISA with microtiter wells. Wells were coated overnight at 4°C with a solution containing the amount of fragment designated on the horizontal axis. (*A*) Antibodies to glomerular proteoglycan; (*B*) antibodies to EHS proteoglycan.

Discussion

Studies from several laboratories (Kanwar et al., 1981; Stow et al., 1983; Parthasarathy and Spiro, 1984; Kanwar et al., 1984; Klein et al., 1986; Edge and Spiro, 1987) show that the heparan sulfate containing proteoglycan from glomerular basement membrane is smaller than the EHS proteoglycan (Hassell et al., 1986). Some of the smaller size is due to the presence of smaller size heparan sulfate side chains on the glomerular proteoglycan. Side chains, it should be noted, are posttranslational modifications and their size is probably determined more by the glycosaminoglycan synthesizing system in the cell than by the precursor protein acceptor (Ledbetter et al., 1985). The relationship between proteoglycans can be more accurately established by characteristics, such as size and immunological determinants of their core proteins or better still, of their precursor proteins.

We have been using the EHS tumor as a model system to characterize the core protein of a basement membrane proteoglycan which contains heparan sulfate side chains. This proteoglycan is synthesized from a M_r = 400,000 precursor

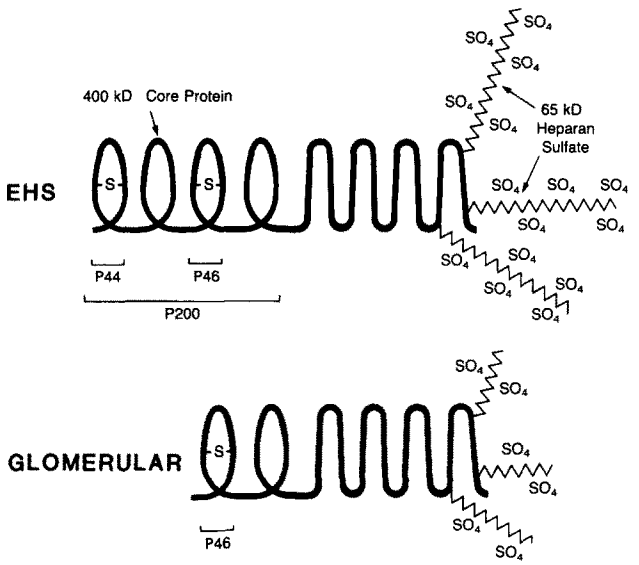

Figure 7. Structural models of EHS and glomerular proteoglycans. The model of the EHS proteoglycan is based on peptide mapping (Ledbetter et al., 1987) and electron microscope visualization (Laurie et al., 1987) as described in the discussion. The results of the studies in this report indicate the glomerular proteoglycans are derived from the same large precursor protein as the EHS proteoglycan but that is proteolytically processed to a smaller core protein containing the P46 region but not the P44 region of the EHS proteoglycan.

protein (Ledbetter et al., 1985) and consists of a M_r = 400,000 core protein containing three heparan sulfate side chains of M_r = 65,000 (Hassell et al., 1985*b*; Kato et al., 1985). The core protein is divided equally into a trypsin sensitive domain containing the side chains and a trypsin resistant domain (P200) containing two V8 protease resistant regions of M_r = 44,000 (P44) and M_r = 46,000 (P46) (Ledbetter et al., 1986). A structural model of the EHS proteoglycan showing the location of the P200, P44 and P46 fragments within the M_r = 400,000 core protein has been proposed (Ledbetter et al., 1986). Recent electron microscopic examination of this proteoglycan shows the side chains extending from one end of the core protein (Laurie et al., 1988; Paulsson et al., 1987; Yurchenco et al., 1987) and this model of the EHS proteoglycan is now redrawn (Fig. 7) for comparison with the glomerular proteoglycan.

The experiments in this study, using antibodies to EHS proteoglycan, show glomerular cells synthesize the same size (M_r = 400,000) precursor protein known to be converted to the major heparan sulfate proteoglycan in EHS cells (Ledbetter et al., 1985). These antibodies also detected heparan sulfate proteoglycan in extracts of glomeruli and glomerular basement membranes that have core proteins ranging in size from M_r = 150,000 to 400,000 with a M_r = 250,000 species being most prevalent. It is likely that this large precursor protein synthesized by glomerular cells is used for the production of glomerular heparan sulfate proteoglycan and that proteolytic processing produces proteoglycans with a range of core protein sizes. The relatively more diffuse core protein bands compared to the sharper precursor protein band probably reflects greater variations in posttranslational modifications such as proteolytic processing and oligosaccharide addition, of the core proteins. Furthermore, antibodies to the glomerular proteoglycan confirm the immunological cross reaction between EHS and glomerular proteoglycans. These antibodies immunoprecipitated the M_r = 400,000 precursor protein synthesized by EHS cells and reacted with most of the proteolytic fragments of the EHS proteoglycan, including the P46 fragment but not the P44 fragment. It is possible that the glomerular proteoglycan used as an antigen was proteolytically clipped in the P200 domain of the M_r = 400,000 precursor protein, either before or after heparan sulfate addition, to produce a proteoglycan with a core protein containing the P46 determinants but lacking the P44 fragment as illustrated in Fig. 7.

The antibodies to the EHS proteoglycan detected heparan sulfate proteoglycans with core proteins of M_r = 250,000 and larger from glomeruli, and cores of M_r = 250,000 and smaller from glomerular basement membrane. These observations may indicate that the proteoglycan components isolated from glomerular basement membrane preparations have been proteolytically processed further than those isolated from glomeruli. Alternatively, since it has been shown (Brown et al., 1981) that at least 95% of the $^{35}SO_4$ incorporated into glomeruli is lost during the isolation of glomerular basement membrane, the appearance of these smaller core proteins and glycoproteins may be due instead to the selective loss of the proteoglycans with the larger core proteins during the procedure used to isolate the glomerular basement membrane.

The size of the core proteins found for glomerular heparan sulfate proteoglycans in this report are considerably larger than the M_r = 18,000 reported by Kanwar et al. (1984) but only slightly larger than the M_r = 143,000 recently reported by Edge and Spiro (1987). The differences are likely to be technical. Firstly, to reduce tissue handling, we isolated proteoglycans directly from glomeruli and these proteoglycans proved to have the largest core proteins. Secondly, the proteoglycans were isolated by DEAE-column chromatography only, thereby reducing either the possibility of additional proteolysis or the selection of one form of proteoglycan during purification. Finally, although the proteoglycans were not rigorously purified, detection of the core proteins relied upon the specificity of both the heparitinase and an affinity purified antibody which has been shown to immunolocalize only to basement membranes in tissue sections and immunoprecipitate only one precursor protein.

An unexpected finding of this study was the presence of M_r = 200,000 and 150,000 glycoproteins in glomeruli and glomerular basement membrane that reacted with the antibody to the EHS proteoglycan. These immunologically related glycoproteins are also likely produced by proteolytic processing of the M_r = 400,000 precursor protein and would not be detected in other studies, where core proteins are defined only by the presence of glycosaminoglycan side chains. An analogous situation exists in cartilage where a fragment of the cartilage proteoglycan containing a functional hyaluronic acid–binding region but not glycosaminoglycan side chains has been identified as a component of the cartilage matrix (Roughley et al., 1985; Roughley and Mort, 1986). The glycoprotein fragments from the core protein of the glomerular proteoglycan may have functional properties of their own as well.

The presence of proteoglycan in basement membranes was first suggested by histological studies showing staining of

basement membranes with cationic dyes that bind glycosaminoglycans (Bernfield and Banerjee, 1972; Trelstad et al., 1974; Caulfield and Farquhar 1976). Heparan sulfate proteoglycans isolated from different basement membranes, however, have been shown to range in size from large (M_r = 600,000) to small (M_r = 130,000) (for reviews see Hassell et al., 1986; Poole, 1986; Gallagher et al., 1986). Antibodies prepared to date against these apparently different proteoglycans localize to all basement membranes (Hassell et al., 1980; Stow et al., 1985; Fenger et al., 1984; Eldridge et al., 1986; Brenchley, P. E., M. A. J. Dickinson, and J. C. Anderson, manuscript in preparation). These antibodies recognize only one (M_r = 400,000) precursor protein (Ledbetter et al., 1985; Hassell et al., 1985*a*; Wewer et al., 1985; Eldrich et al., 1986; Singer et al., 1987; this report) from a variety of cell types. Furthermore, metabolic studies with basement membrane producing cells in culture show that at least a portion of the population of small proteoglycans are derived from larger ones (Ledbetter et al., 1985; Kinsella and Wight, 1985). Thus, proteolytic processing of the M_r = 400,000 precursor protein to a smaller core protein of a proteoglycan may be a recurrent theme in the biosynthesis of basement membrane proteoglycans. This processing, or core protein "tailoring," would produce a proteoglycan which fits the functional requirements of an endothelial, epithelial, muscle, adipose, or neuronal basement membrane. Removing selected regions of the core might affect the proteoglycans interaction with other basement membrane components, change its matrix location and possibly modify its function as well.

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