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## Abstract

Using the technique of somatic cell fusion, we produced monoclonal antibodies to collagenase-digested human glomerular basement membrane (GBM). Fourteen monoclonal antibodies which reacted with normal human kidney in indirect immunofluorescence (IIF) studies were produced. An analysis of the binding patterns indicated that the antigens recognized could be divided into six broad groups. Monoclonal antibody B3-H10 (Group 1) reacted with only GBM in a fine granular pattern. A5-B12 and B5-C2 (Group 2) reacted with GBM and peritubular capillary in a linear pattern. B2-A12 (Group 3) reacted with only epithelial cells. Al-C9 and A4-E2 (Group 4) showed a mesangial pattern in glomerulus and a lineal pattern in tubular basement membrane (TBM), Bowman's capsule and peritubular capillary. A1-E1, A1-E11, A2-E6, A3-B6, A4-F8 and B5-H2 (Group 5) recognized determinants common to GBM, TBM, Bowman's capsule and/or peritubular capillary. A3-F1 and B5-E10 (Group 6) reacted with TBM and Bowman's capsule. The staining pattern of B3-H10 (Group 1) was characteristic because it was not linear, but finely granular along the GBM. The staining pattern of B2-A12 (Group 3) was also characteristic because only epithelial cells were stained, and processes of epithelial cells were observed as fine fibrils. To the best of our knowledge, these two types of monoclonal antibodies have not been reported previously.

**KEYWORDS:** monoclonal antibodies, human glomerular bacemant membrance

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**—— BRIEF NOTE ——**

# PRODUCTION OF MONOCLONAL ANTIBODIES TO HUMAN GLOMERULAR BASEMENT MEMBRANE

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*Abstract.* Using the technique of somatic cell fusion, we produced monoclonal antibodies to collagenase-digested human glomerular basement membrane (GBM). Fourteen monoclonal antibodies which reacted with normal human kidney in indirect immunofluorescence (IIF) studies were produced. An analysis of the binding patterns indicated that the antigens recognized could be divided into six broad groups. Monoclonal antibody B3-H10 (Group 1) reacted with only GBM in a fine granular pattern. A5-B12 and B5-C2 (Group 2) reacted with GBM and peritubular capillary in a linear pattern. B2-A12 (Group 3) reacted with only epithelial cells. Al-C9 and A4-E2 (Group 4) showed a mesangial pattern in glomerulus and a lineal pattern in tubular basement membrane (TBM), Bowman's capsule and peritubular capillary. Al-E1, Al-E11, A2-E6, A3-B6, A4-F8 and B5-H2 (Group 5) recognized determinants common to GBM, TBM, Bowman's capsule and/or peritubular capillary. A3-F1 and B5-E10 (Group 6) reacted with TBM and Bowman's capsule. The staining pattern of B3-H10 (Group 1) was characteristic because it was not linear, but finely granular along the GBM. The staining pattern of B2-A12 (Group 3) was also characteristic because only epithelial cells were stained, and processes of epithelial cells were observed as fine fibrils. To the best of our knowledge, these two types of monoclonal antibodies have not been reported previously.

*Key words :* monoclonal antibodies, human glomerular basement membrane

In recent years, monoclonal antibodies against a great variety of antigens have been produced by cell fusion technology. In the field of nephrology, monoclonal antibodies to brush border (1), rat glomerular antigens (2), human glomerular basement membrane (3) and human glomerular cells (4, 5) have been reported.

In this work, we produced monoclonal antibodies to human GBM and investigated the heterogeneity of GBM antigen.

## *Materials and Methods*

*Preparation of collagenase-solubilized human GBM.* Human kidneys were obtained from autopsy cases without obvious renal disease. Glomeruli were isolated by a modification of the method of Spiro as described previously (6). The abscence of tubular contamination was

confirmed under a phase contrast microscope. Glomeruli were suspended in 1 M saline and sonicated until no cells remained adherent to basement membrane particles. Four hundred mg of the particles were washed in distilled water, lyophilized, and resuspended in 0.05 M Tris-HCl buffer, pH 7.5, in the presence of 0.01 M $CaCl_2$. The particles were incubated with 25 mg of Type 1A collagenase (Sigma) for 24 h at 37℃ while shaking. The mixture was centrifuged at 20,000 × g for 45 min, and the supernatant containing collagenase-solubilized human GBM was fractionated on Sephadex G 100 columns. A fraction of GBM with a molecular weight of approximately 70,000 daltons was used for immunization.

*Immunization, cell fusion and cloning.* Two CBA mice were injected intracutaneously with 0.5 mg of the fractionated human GBM homogenated in complete Freund's adjuvant. Immunization was repeated four times weekly. Three days after the last injection, the mice were killed and the spleen cells were fused with mouse myeloma line P3-NSAl-Ag4-1 (NSl) cells using the standard protocol of Köhler and Milstein (7).

Culture supernatants were tested for the presence of specific antibodies by IIF, and cells of interest were subcloned by limiting dilution onto a feeder layer of mouse thymocytes. Seven to fourteen days after the initial cloning, supernatant from each limiting dilution was tested by IIF, and the cells were again subcloned.Seven to fourteen days after the second cloning, a third cloning was done in the same manner. The IIF was performed with fluorescein-conjugated goat anti-mouse IgG on normal human kidney slices.

## *Results and Discussion*

The IIF staining patterns of the fourteen different monoclonal antibodies on frozen sections of normal human kidney are given in Table 1. According to the selective reactivity with morphologically distinct parts of the nephron, six groups

TABLE 1. BINDING PATTERNS OF MONOCLONAL ANTIBODIES AGAINST HUMAN RENAL BASEMENT MEMBRANES BY INDIRECT IMMUNOFLUORESCENCE.

| Group | Monoclonal antibody | GBM | Mesangium | Epithelium | Peritubular capillary | TBM | Bowman's capsule |
|---|---|---|---|---|---|---|---|
| 1 | B3-H10 | ++ (*) | − | − | − | − | − |
| 2 | A5-B12 | +++(L) | − | − | +++ | − | − |
| | B5-C 2 | +++(L) | − | − | +++ | − | − |
| 3 | B2-A12 | ± | − | +++ | ± | − | − |
| 4 | A1-C 9 | + (L) | ++ | − | + | +++(L) | +++ |
| | A4-E 2 | + | +++ | − | +++ | +++ | +++ |
| 5 | A1-E 1 | ++ (L) | − | − | − | + (L) | +++ |
| | A1-E11 | +++(L) | − | − | ++ | ++ (L) | ± |
| | A2-E 6 | ++ (L) | − | − | − | ++ (L) | + |
| | A3-B 6 | +++(L) | − | − | ++ | + (L) | − |
| | A4-F 8 | ++ (L) | − | − | ++ | ++ (L) | ++ |
| | B5-H 2 | ++ (L) | − | − | − | + (L) | − |
| 6 | A3-F 1 | ± | − | − | − | ++ (L) | +++ |
| | B5-E10 | − | − | − | − | +++(L) | +++ |

* : fine granular pattern L : linear pattern

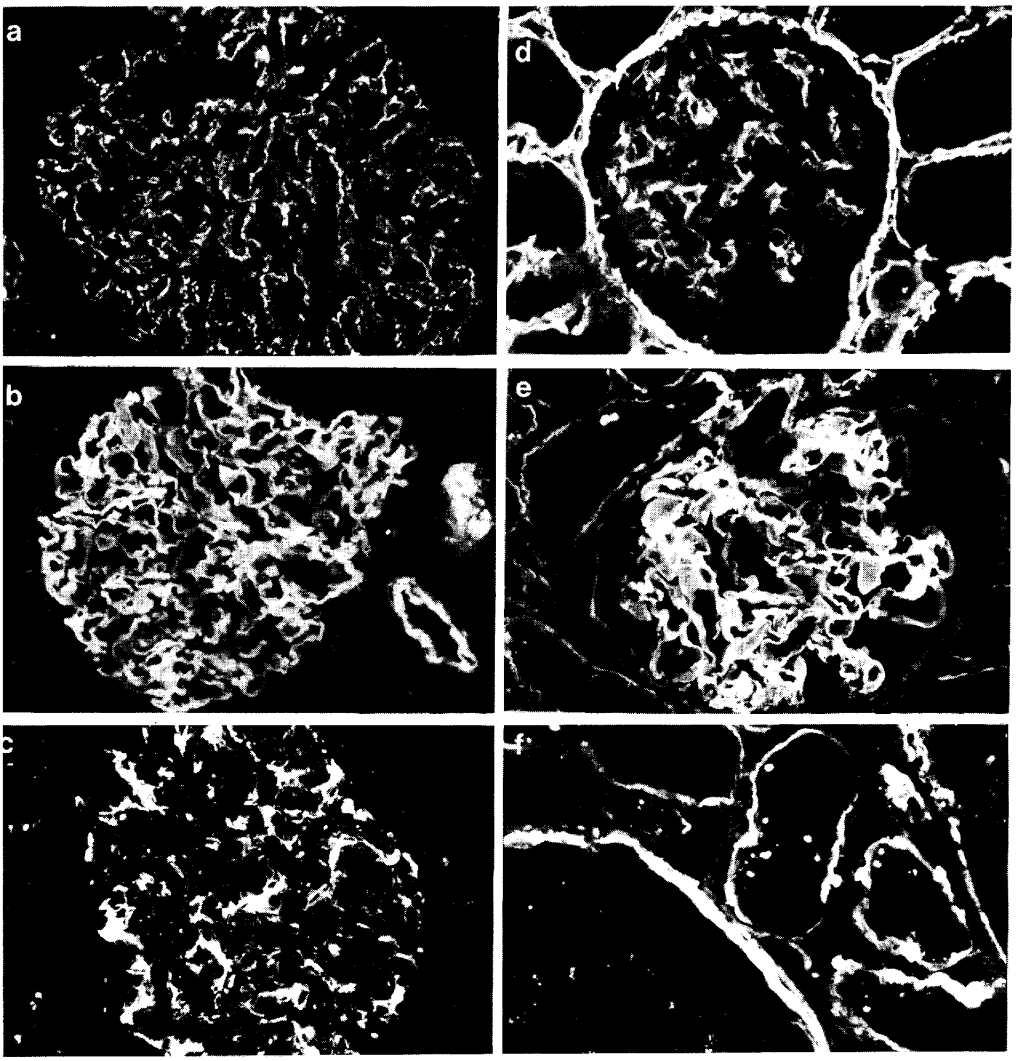


Fig. 1. Indirect immunofluorescence staining of monoclonal antibodies on normal human kidney sections. (a) B3-H10 (Group 1) stains only GBM in a fine granular pattern, but not TBM, Bowman's capsule and peritubular capillary. (× 200) (b) B5-C2 (Group 2) reacts with GBM and peritubular capillary in a linear pattern. (× 200) (c) B2-A12 (Group 3) reacts with only epithelial cells with fine fibrils. (× 200) (b) A1-C9 (Group 4) shows a mesangial pattern in glomerulus and a linear pattern in TBM, Bowman's capsule and peritubular capillary. (× 200) (e) A1-E11 (Group 5) reacts in a linear pattern with GBM, TBM, peritubular capillary and Bowman's capsule. (× 200) (f) B5-E10 (Group 6) reacts chiefly with TBM and Bowman's capsule. (× 200)

of monoclonal antibodies were distinguished. Monoclonal antibody B3-H10 (Group 1) was found to stain only GBM in a fine granular pattern (Fig. 1-a). A5-B12 and B5-C2 (Group 2) reacted with GBM and peritubular capillary in a linear pattern (Fig. 1-b), B2-A12 (Group 3) reacted with only epithelial cells (Fig.

1-c). A1-C9 and A4-E2 (Group 4) showed a mesangial pattern in glomerulus and a linear pattern in TBM, Bowman's capsule and peritubular capillary (Fig. 1-d). A1-E1, A1-E11, A2-E6, A3-B6, A4-F8 and B5-H2 (Group 5) recognized determinants common to GBM, TBM, Bowman's capsule and/or peritubular capillary (Fig. 1-e). A3-F1 and B5-E10 (Group 6) reacted with TBM and Bowman's capsule (Fig. 1-f).

Among these monoclonal antibodies, B3-H10, A5-B12, B5-C2 and B2-A12 (Groups 1, 2 and 3) were shown to define the antigens primarily expressed on glomerulus. Especially, the staining pattern of B3-H10 (Group 1) was characteristic because it was not linear, but finely granular along the GBM. The staining pattern of B2-A12 (Group 3) was also characteristic because only epithelial cells were stained, and processes of epithelial cells were observed as fine fibrils. To the best of our knowledge, these two types of monoclonal antibodies have not been reported previously. The distribution of A1-C9 and A4-E2 (Group 4) appeared similar to that of type IV collagen as revealed by Michael *et al.* (5) and Scheinman *et al.* (8).

Studies of these antibodies may contribute to the understanding of GBM and TBM antigenecities in normal kidney and different forms of glomerulonephritic kidney in man.

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