Brief Definitive Report

CHARACTERIZATION OF A 280-kD PROTEIN RESTRICTED TO THE COATED PITS OF THE RENAL BRUSH BORDER AND THE EPITHELIAL CELLS OF THE YOLK SAC

Teratogenic Effect of the Specific Monoclonal Antibodies

BY D. SAHALI,* N. MULLIEZ,§ F. CHATELET,‡ R. DUPUIS,§ P. RONCO,* AND P. VERROUST*

*From the *Institut National de la Santé et de la Recherche Médicale, Unité 64 and ‡Laboratoire d'Histologie et Cytologie Pathologiques, Hôpital Tenon, Paris 75020; and the §Département d'Embryologie, Hôpital Saint Antoine, Paris, France 75571*

Within the brush border (BB) of proximal tubule cells (PTC), the intermicrovillar area connecting the base of the microvilli constitutes a distinct microdomain characterized by the presence on the cytoplasmic side of the membrane of an extensive clathrin coat (1). Kerjaschki and Farquhar (2) and Chatelet et al. (3) have shown that a 330-kD glycoprotein (gp), initially described as the nephritogenic antigen of Heymann's nephritis, is concentrated on the luminal aspect of these areas, at variance with other BB proteins such as maltase (4), which are distributed over the entire surface of the microvilli. To our knowledge, gp330 is the only membrane gp confined to the intermicrovillar domain (IMVD). It is also expressed in coated pits of glomerular epithelial cells, pneumocytes type II, epididymis, and epithelial cells of the visceral yolk sac (VYS) (5, 6), but its function remains unknown.

This paper describes a 280-kD BB protein, defined by mAbs raised against rat renal BB, which is only found in the IMVD of the PTC and on the apical domain of epithelial cells of the VYS. When injected to pregnant rats, the mAbs induce embryonic resorption and/or fetal abnormalities, suggesting that the 280-kD protein plays a key role in endocytosis-related cell function.

Materials and Methods

Antibodies. The two mAbs reported in this study (F5/75, an IgG1 with a pI of 6.8–7.6, and F5/46, an IgG1 with a pI of 8.1–8.5) were obtained by immunization of mice against rat BB (7). They were analyzed in relation to mAb F1/12 and polyclonal antibodies specific for gp330 (8), and mAb F2/180 reactive with the 300-kD maltase.

Characterization of the 280-kD Antigen. The antigen identified by mAb F5/75 and F5/46 on tubular BB was identified by immunoprecipitation of radiolabeled BB membrane vesicles (BBMV) (7) and immunoadsorption techniques. Purified mAbs F5/75 and F5/46, as well as mAbs F1/12 and F2/180, were individually coupled to Sepharose 4B (Pharmacia Fine Chemicals, Velizy, France). All steps involving antigenic preparations and immunoadsorption were carried out in the presence of 1 mM PMSF and 2 mM benzamidine. BBMV (7, 8) and yolk sacs dissected at 19 d of gestation were washed extensively in PBS and solubilized in 1% Triton X100. 1–2 ml of each immunoabsorbent was incubated with either 20 mg of solubilized BBMV, or with the material from 10 yolk sacs. After extensive

TABLE I

Effect of Antibodies to p280 and gp330 on Embryonic Development

Antibody	Specificity	Dose	Litters	Eggs	Embryonic resorption (%)	Surviving fetuses*	
						Total number	Number with malformations (%)
		mg					
mAb 75	p280	5	10	136	65 (47.7)	71	51 (71.8)
		5‡	4	55	18 (32.7)	37	24 (64.8)
		10	4	60	59 (98.3)	1	1
mAb 46	p280	3	5	74	22 (29.7)	52	11 (21.2)
		4	2	13	10 (76.9)	3	3
		5	5	72	72 (100)	0	—
Mix. mAb‖	gp330	1–5	3	44	0	44	0
Rabbit	gp330	0.25–5¶	4	58	0	58	0
LPC1	—	2.5	4	46	5 (11)	41	0

* The following types of malformations were observed in a total of 90 abnormal embryos: eye defects (anophtalmia, microphtalmia), 90%; testis ectopia (25/50 ♂), 50%; external-ear abnormalities, 42.2%; hydronephrosis, 37.8%; neural tube defects, 25.5%; Muller duct agenesis (9/40 ♀), 22.5%; tail abnormalities, 21.1%; hydrocephaly, 21.1%; cardiovascular defects, 13.3%; kidney agenesis, 13.3%; diaphragmatic hernia or eventration, 12.2%; celosomia, 4.4%.

Pregnant rats were injected at day 9 with mAb containing ascites or anti-gp330 rabbit serum.

‡ Purified antibody.

‖ Mixture of equal amounts of four mAbs specific for distinct epitopes on gp330 (8).

¶ ml.

sequential washing with PBS, 2 M NaCl, and H_2O, the bound material was eluted with diethylamine (8), concentrated by lyophilization, and analyzed by SDS-PAGE.

Immunohistochemical Binding of mAb 75 and mAb 46. Indirect immunofluorescence (IIF) binding of mAb F5/75 and F5/46 was studied on 2-μm acetone-fixed frozen sections of kidney, lung, liver, spleen, epididymis, gut, and yolk sac using species-specific fluorescein-labeled anti–mouse IgG (7). Binding at the ultrastructural level on renal BB and yolk sac was studied using preembedding immunoperoxidase techniques (3).

Teratogenicity Experiments. The effects of antibodies to p280 on embryonic development (embryonic resorption and induction of fetal malformations) were studied by injection to rats 9 d after the beginning of gestation of antibodies listed in Table I. In vivo binding of the injected antibody was assessed by IIF on yolk sacs and fetuses, 24–48 h after injection.

Results and Discussion

Characterization of a New Coated Pit–restricted 280-kD BB Protein. Indirect immunofluorescence of kidney sections showed that the mAbs F5/75 and F5/46 bound selectively proximal tubule BB (Fig. 1 *A*). Glomeruli were not stained and binding predominated in the deep areas of the cortex. By contrast, mAb F1/12 specific for gp330 binds equally well on all proximal tubule sections and can be localized in glomeruli (7, 8). At the ultrastructural level (Fig. 1 *B*) binding of F5/75 or F5/46 predominated in the IMVD, but could occasionally be more diffuse in some tubular segments. This pattern is very similar to that previously reported for gp330 (2, 3) and contrasts with the diffuse distribution of maltase over the entire microvilli (4).

The molecular mass of the antigen identified by F5/75 and F5/46 was defined using immunoprecipitation of radiolabeled BBMV and immunoadsorption techniques. As shown in Fig. 2, the two mAbs were able to bind an antigen with a

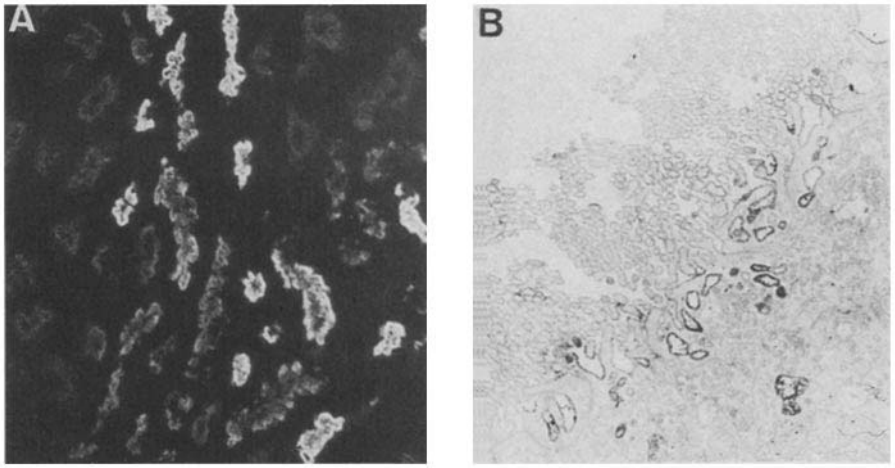

FIGURE 1. Expression within the renal parenchyma of p280. (*A*) Indirect immunofluorescence. Note irregular distribution of p280. Original magnification: × 250. (*B*) Immunoperoxidase analysis at the ultrastructural level. Note that p280 is essentially expressed within the IMVD. Original magnification: × 4,400.

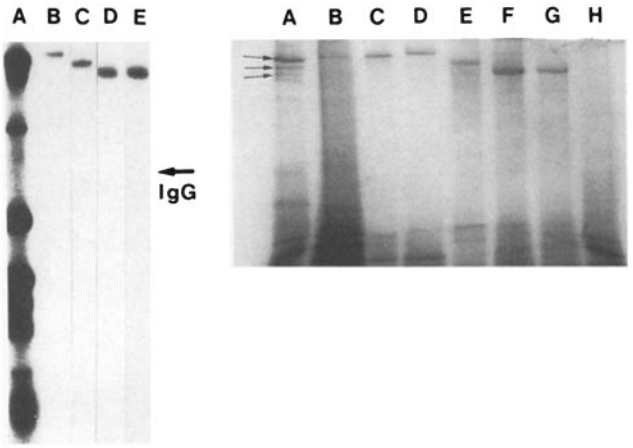

FIGURE 2. Characterization of the antigen identified by mAb F5/75 and mAb F5/46. (*Left panel*) Immunoprecipitation of radiolabeled BBMV: autoradiograph of SDS-PAGE. (*A*) Radiolabeled BBMV; (*B–E*) material immunoprecipitated by anti-gp330 F1/12 (*B*), anti-maltase F2/180 (*C*), F5/46 and F5/75 (*D* and *E*). (*Right panel*) Immunoaffinity chromatography of solubilized BBMV and YS: proteins separated by SDS-PAGE were stained by Coomassie blue. (*A* and *B*) Solubilized BBMV (*A*) and yolk sac (*B*); (*C* and *D*) material purified from BBMV (*C*) and YS (*D*) using S4B-F1/12; (*E*) material purified from BBMV using S4B-F2/180; (*F* and *G*) material purified from BBMV (*F*) and YS (*G*) using S4B-F5/46; (*H*) control immunoadsorption using S4B-LPC1. Arrows in lane *A* indicate from top to bottom migration of gp330, maltase (300 kD), and p280.

molecular mass estimated to 280 kD, clearly lower than those of both the Heymann's nephritis antigen (330 kD) and maltase (300 kD).

Extrarenal Distribution of the 280-kD Protein. Survey by IIF of various organs showed that the epithelium of the VYS (Fig. 3*B*) was the only tissue stained by mAbs F5/46 and F5/75. These results are distinct from those obtained for gp330, which in addition to kidney and VYS (Fig. 3, *E* and *F*) is detectable on epididymis, pneumocytes type II (5), and embryonic tissue at day 10 of gestation (Fig. 3*E*). At the ultrastructural level, both gp330 and p280 were associated with the clathrin-coated vesicular system of the VYS (Fig. 3*A*). gp330 (Fig. 3*G*) was

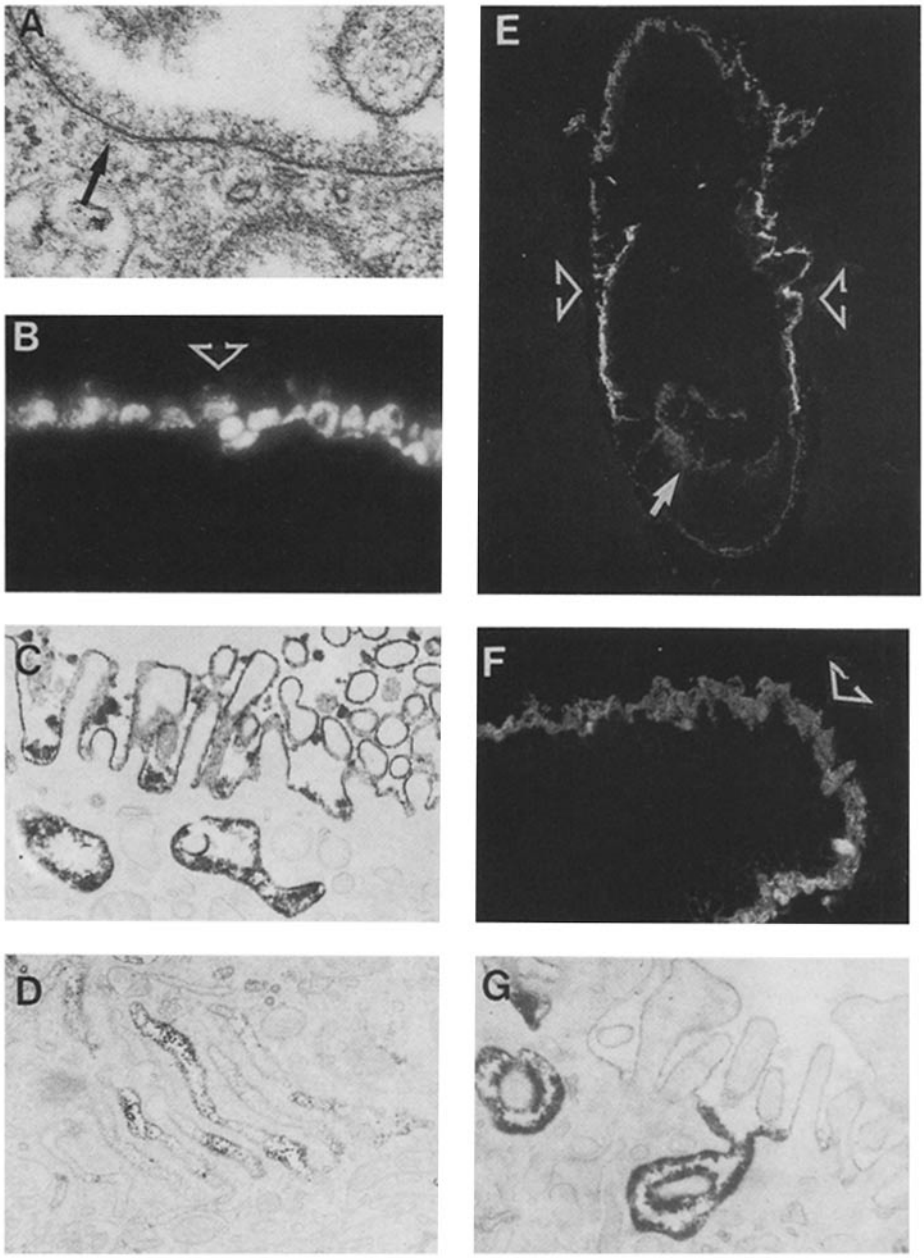

FIGURE 3. Comparative expression on YS-epithelial cells of p280 and gp330. (*A*) High magnification electron micrograph of a VYS epithelial cell showing the clathrin coat on the cytoplasmic aspect of an apical vesicle (*arrow*). Note abundant glycocalix on the inner aspect. Original magnification: × 20,000. (*B*, *E*, and *F*) Indirect immunofluorescence using anti-p280 F5/75 (*B*) or anti-gp330 F1/12 (*E* and *F*). The two mAbs bind to VYS epithelial cells (*arrowheads*). Note that, in addition, anti-gp330 bind to embryonic structures (*arrows*). Original magnification: (*B* and *F*) × 400; (*E*) × 150. (*C*, *D*, and *G*) Immunoperoxidase analysis at the ultrastructural level using F5/46 anti-p280 (*C* and *D*) and F1/12 anti-gp330 (*G*). Note that gp330 and p280 are both expressed on the inner aspect of clathrin coated vesicles. gp330 is restricted to membrane coated pits (*G*) whereas p280 (*C*) has a more diffuse expression. (*D*) Golgi apparatus stained for p280. Original magnifications: × 20,000.

found within clathrin-coated vesicles and in the intermicrovillar areas of the cell membrane. p280 (Fig. 3*C*) was detectable within the same coated vesicles but distribution on the cell surface was diffuse, involving both microvilli and intermicrovillar areas. Uncoated vesicles, lysosomes, and the apical canalicular system were never stained by either of the two antibodies.

The results of immunoadsorption experiments (Fig. 2) show that the proteins immunopurified by anti-gp330 and anti-p280 antibodies from YS and BB comigrate with identical apparent molecular weights.

Teratogenesis Experiments. Table I shows that either of the two mAbs to anti-p280 induced in a dose-dependent manner a high frequency of embryonic resorption and fetal malformations in the surviving fetuses. By contrast, monoclonal or polyclonal antibodies specific for gp330 had no effect. 24 h after injection, anti-gp330 were localized on VYS as well as in the mother's glomeruli (8) and in embryonic structures, whereas anti-p280 were only detectable on VYS, thus suggesting that anti-p280–induced embryonic abnormalities are not related to immunological disease of the mother or to antibody binding to embryonic structures.

The induction of birth defects by antikidney antibodies was described by Brent (9) and confirmed by others (10, 11). Our observations provide new data in two domains: (*a*) Leung (11) isolated a 340-kD BB antigen, reported as diffuse on BB microvilli, which elicited teratogenic antibodies reactive with epithelial cells of the VYS, but the antigen isolated from the latter structure (12) consisted of two peptides of 30 and 60 kD. Our results show that the teratogenic mAbs prepared in our laboratory identify a 280-kD protein from both yolk sac and BB. (*b*) The rodent yolk sac plays a major role in providing nutriments to the embryo (13), essentially via catabolism of proteins taken up by fluid-phase and/or receptor-mediated endocytosis. Alteration of this process by chemical means (reviewed in references 12, 13) or the use of antibodies (14) induces abnormal fetal development. Our data indicate that the key step involves interaction with, and presumably blockade of, the function of a protein associated with the clathrin-coated vesicular system and thus probably involved in receptor-mediated endocytosis. This effect is highly specific since antibodies to another coated pit protein are inefficient. Moreover, it can be obtained using mAbs, suggesting the presence on p280 of epitopes critical for its physiological function.

The relevance of this observation in man is at present unknown. Proteins immunologically related to murine p280 are expressed by the human BB (our unpublished data) and possibly by fetal envelopes. The possibility thus exists that an immune response to such a protein may explain some of the spontaneous abortions observed.

Summary

Intermicrovillar areas and apical vesicles characterized by an extensive clathrin coat can be identified in some epithelial cell types. We describe a 280-kD protein, characteristic of these areas in the proximal tubule brush border and epithelial cells of the visceral yolk sac. When injected to 9-d pregnant rats, mAbs to the 280-kD protein regularly induced fetal resorption and/or malformations. Antibodies to a 330-kD protein that is also coated-pit–restricted had no effect. Our

observations point to a key function for p280 and suggest that immunity to specific constituents of the receptor-mediated endocytotic system may be involved in the induction of fetal abnormalities.

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References

1. Rodman, J. S., D. Kerjaschki, E. Merisko, and M. G. Farquhar. 1984. Presence of an extensive clathrin coat on the apical plasmalemma of the rat kidney proximal tubule cell. *J. Cell Biol.* 98:1630.
2. Kerjaschki, D., and M. G. Farquhar. 1983. Immunocytochemical localization of the Heymann nephritis antigen (gp330) in glomerular epithelial cells of normal Lewis rats. *J. Exp. Med.* 157:667.
3. Chatelet, F., E. Brianti, P. Ronco, J. Roland, and P. Verroust. 1986. Ultrastructural localization by monoclonal antibodies of brush border antigens expressed by glomeruli: I. Renal distribution. *Am. J. Pathol.* 122:500.
4. Kerjaschki, D., L. Noronha Blob, B. Sacktor, and M. G. Farquhar. 1984. Microdomains of distinctive glycoprotein composition in the kidney proximal tubule brush border. *J. Cell Biol.* 98:1505.
5. Chatelet, F., E. Brianti, P. Ronco, J. Roland, and P. Verroust. 1986. Ultrastructural localization by monoclonal antibodies of brush border antigens expressed by glomeruli: II. Extrarenal distribution. *Am. J. Pathol.* 122:512.
6. Doxsey, S., D. Kerjaschki, and M. G. Farquhar. 1983. A large membrane glycoprotein is a resident of coated pits of several absorptive epithelia. *J. Cell Biol.* 97:(5, Pt. 2):178*a*. (Abstr.)
7. Ronco, P., C. Melcion, M. Geniteau, E. Ronco, L. Reininger, M. Galceran, and P. Verroust. 1984. Production and characterization of monoclonal antibodies against brush border antigens of the proximal convoluted tubule. *Immunology.* 53:87.
8. Ronco, P., T. J. Neale, C. B. Wilson, M. Galceran, and P. Verroust. 1986. An immunopathological study of a 330 kD protein defined by monoclonal antibodies and reactive with anti-RTEα5 antibodies and kidney eluates from active Heymann nephritis. *J. Immunol.* 136:125.
9. Brent, R. L., E. Averich, and V. A. Drapiewski. 1961. Production of congenital malformations using tissue antibodies. I. Kidney antisera. *Proc. Soc. Exp. Biol. Med.* 106:523.
10. David, G., L. Mercier-Parot, and H. Tuchmann-Duplessis. 1963. Action tératogène d'hétéroanticorps tissulaires. Production de malformations chez le rat par action d'un serum antirein. *C. R. Seances Soc. Biol. Fil.* 157:939.
11. Leung, C. C. K. 1982. Isolation, partial characterization, and localization of a rat renal tubular glycoprotein antigen. *J. Exp. Med.* 156:372.
12. Leung, C. C. K., C. Lee, B. Cheewatrakoolpong, and D. Hilton. 1985. Abnormal embryonic development induced by antibodies to rat visceral yolk-sac endoderm: isolation of the antigen and localization to microvillar membrane. *Dev. Biol.* 107:432.
13. Jollie, W. P. 1986. Review article: ultrastructural studies of protein transfer across rodent yolk sac. *Placenta.* 7:263.
14. Freeman, S. J., R. L. Brent, and J. B. Lloyd. 1982. The effect of teratogenic antiserum on yolk-sac function in rat embryos cultured in vitro. *J. Embryol. Exp. Morphol.* 71:63.