Antibodies to protein tyrosine phosphatase receptor type O (PTPro) increase glomerular albumin permeability ($P_{\text{alb}}$)

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Production and characterization of the rabbit PTPro reagents. mAbs to PTPro (4C3 and P8E7) as well as to podocalyxin (5F7) were produced following immunization of mice with isolated rabbit glomeruli (25). 4C3 appears to bind to the amino acid core of the extracellular domain of PTPro since it binds to the denatured PTPro molecule under reducing conditions (14). Similarly, the interaction between nephrin and phosphoinositide 3-kinase appears to be important in nephrin-mediated actin cytoskeletal rearrangement (35). These and other data support the model that homophilic interactions of the nephrin ectodomain result in phosphorylation of specific nephrin cytoplasmic domain tyrosines, recruitment of the adapter protein Nck, and consequent assembly of actin filaments (8, 13, 28). Events occurring at the slit-junction may well be essential to the control of the filtration barrier.

We postulated that PTPro activity may play a role in determining glomerular macromolecular permeability. Its location on the apical surface of the podocyte and its role in tyrosine phosphorylation make it an attractive candidate. We investigated the effect of antibodies against the extracellular domain (ECD) of PTPro on albumin permeability ($P_{\text{alb}}$) using isolated glomeruli, (18). Certain species-specific monoclonal (mAb) and polyclonal antibodies increased $P_{\text{alb}}$ in both rat and rabbit glomeruli. One mAb that increased $P_{\text{alb}}$ also decreased phosphatase activity while another had no effect on either phenomenon. We conclude that the interaction of certain antibodies against ECD epitopes of PTPro acutely increases glomerular macromolecular permeability, possibly through inactivation of phosphatase activity. Thus tonic activity of PTPro appears to play a role in maintaining normal function of the glomerular filtration barrier.

**Materials and Methods**

**Production and characterization of the rabbit PTPro reagents.** mAbs to PTPro (4C3 and P8E7) as well as to podocalyxin (5F7) were produced following immunization of mice with isolated rabbit glomeruli (25). 4C3 appears to bind to the amino acid core of the extracellular domain of PTPro since it binds to the denatured PTPro molecule under reducing conditions and to the nonglycosylated form of the PTPro molecule expressed by Escherichia coli as a fusion protein and in the cDNA expression system. Cloning data indicate that the 4C3 binding epitope is within fibronectin repeat 3. In contrast, P8E7 mAb does not recognize PTPro under reducing conditions on Western blotting, and it does not recognize the PTPro extracellular domain in bacterial expression systems. We have interpreted these
fibrinectin repeats 2–5 was prepared by PCR and cloned into a vector for expression of the GST fusion protein. The fusion protein was purified by glutathione affinity chromatography and used for preparation of both mAbs and polyclonal antibodies in mice.

**Antibody production and purification.** For polyclonal antibody production, four rabbits were immunized using 100 μg of the affinity-purified PTPro ECD GST fusion protein. Immune rabbit IgG was purified by ammonium sulfate fractionation followed by affinity purification on a rat PTPro ECD GST Sepharose 4B column and eluted with glycine HCl, pH 2.5. For mAb production, two mouse mAbs recognizing rat PTPro extracellular domain fusion protein were generated using standard methods. These antibodies were of the IgG2a isotype. As a control, a mAb designated L11–135, which recognizes rabbit class II (DQ) but not rat class II proteins, was class switched from IgG1 to IgG2a by clonal selection using limiting dilution and an IgG2a-specific ELISA.

**Characterization of antibody binding to rat and rabbit glomeruli.** Rabbit and rat kidney cryostat sections fixed with methanol were used for assaying inhibition of antibodies following incubation with species-specific extracellular domain fusion proteins. To confirm that antibodies were specific, we preincubated mAbs with fusion proteins (10-fold excess of fusion protein by weight for rabbits and equal amount of fusion protein for rats) for 30 min at 20°C. Following a blocking step using 10% human serum antibody, preparations were added at 2 μg/100 μl volume and divided a rotor. Beads were then washed four times with TBS containing 4% BSA and assayed for phosphatase activity using p-nitrophenylphosphate as a substrate (25). As described above, the same extracts were also analyzed by Western blotting using mAb 4C3 to quantify the amount of PTPro protein in the immunoprecipitates to confirm that mAbs 4C3 and P8E7 precipitated the same amount of PTPro protein. PTPro extracellular glutathione-S-transferase (GST) fusion protein was produced as previously described (25).

**Antibody effect on phosphatase activity of PTPro.** Briefly, rabbit glomeruli were isolated following cold perfusion and iron embolization as previously described. Isolated glomeruli were suspended in Ringer buffer containing 4% BSA and 25 μM NDidrophenylphosphate, and 2) the 32P-labeled myelin basic protein as substrate as previously described (25).

**Statistical analysis.** Values for P<sub>α</sub> are expressed as average ± SD, and n indicates the number of experimental animals studied, unless indicated otherwise. Comparisons among groups of animals were made using ANOVA. A P value < 0.05 was accepted as significant.

**RESULTS**

Specificity of binding of antibodies directed against the ECD of rabbit and rat glomerular PTPro. mAb 4C3 against rabbit PTPro bound to rabbit tissue but not to rat tissue. Binding was blocked by preincubation with rabbit ECD fusion protein. The
ANTIBODIES TO PTPRO INCREASE $P_{\text{alb}}$

Table 1. Reagents used in studies of $P_{\text{alb}}$ of rabbit and rat glomeruli

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Specificity</th>
<th>Concentration, µg/ml</th>
<th>Incubation, min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rabbit Glomeruli</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Antibodies to rabbit PTPRO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4C3</td>
<td>Anti-rabbit PTPRO ECD mAb, IgG1</td>
<td>5, 10, 25</td>
<td>60, 120</td>
</tr>
<tr>
<td>P8E7</td>
<td>Anti-rabbit PTPRO ECD mAb, IgG1</td>
<td>25</td>
<td>60, 120</td>
</tr>
<tr>
<td>Irrelevant antibodies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB5</td>
<td>Control mAb, anti-human GBM, laminin binding</td>
<td>25</td>
<td>60, 120</td>
</tr>
<tr>
<td>SF7</td>
<td>Control mAb, IgG1 isotype, anti-podocalyxin</td>
<td>55</td>
<td>60, 120</td>
</tr>
<tr>
<td>Fusion proteins</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit PTPRO FP</td>
<td>GST fusion protein of rabbit PTPRO ECD</td>
<td>250</td>
<td>60</td>
</tr>
<tr>
<td>Rabbit podocalyxin FP</td>
<td>GST fusion protein of rabbit podocalyxin ECD</td>
<td>250</td>
<td>60</td>
</tr>
<tr>
<td><strong>Rat Glomeruli</strong></td>
<td></td>
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</tr>
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<td>Antibodies to rat PTPRO</td>
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<tr>
<td>Rat PTPRO polyclonal Ab</td>
<td>Polyclonal Ab to rat PTPRO ECD</td>
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<td>60</td>
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<tr>
<td>1B4</td>
<td>Anti-rat PTPRO ECD mAb, IgG2a isotype</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>1D1</td>
<td>Anti-rat PTPRO ECD mAb, IgG2a isotype</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>Irrelevant antibodies</td>
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<td></td>
<td></td>
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<tr>
<td>Preimmune rabbit serum</td>
<td>Control rabbit serum</td>
<td>0.5–5</td>
<td>10–60</td>
</tr>
<tr>
<td>4C3</td>
<td>Anti-rabbit PTPRO ECD mAb, IgG2a isotype</td>
<td>5</td>
<td>60</td>
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<tr>
<td>BB5</td>
<td>Control mAb, anti-human GBM, laminin binding</td>
<td>5</td>
<td>60, 120</td>
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<tr>
<td>L11–135</td>
<td>Control mAb, IgG2a isotype, rabbit class II (DQ) proteins</td>
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<td>60</td>
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<tr>
<td>Fusion proteins</td>
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</tr>
<tr>
<td>Rat PTPRO FP</td>
<td>GST fusion protein of rat PTPRO ECD</td>
<td>5</td>
<td>60</td>
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$P_{\text{alb}}$, albumin permeability; PTPRO, protein tyrosine phosphatase receptor type O; mAb, monoclonal antibody; ECD, extracellular domain; GBM, glomerular basement membrane; FP, fusion protein; GST, glutathione-S-transferase.

Effect of mAb binding on PTPRO phosphatase activity. The immunoprecipitates of rabbit glomerular proteins prepared using 4C3 and P8E7 contained the same amount of the PTPRO protein. However, the 4C3 immunoprecipitate showed approximately half the phosphatase activity compared with the P8E7 immunoprecipitate (Fig. 2). The average phosphatase activity in the 4C3 immunoprecipitate was $45 \pm 8\%$ of that in the P8E7 immunoprecipitate ($n = 8$, $P < 0.01$). Thus binding of mAb 4C3 to the ECD of PTPRO reduces phosphatase activity.

Effect of antibodies against the ECD of PTPRO on $P_{\text{alb}}$ of isolated rabbit glomeruli. Incubation with 4C3 for 10 min increased $P_{\text{alb}}$, an effect seen for up to 120 min, the longest time point studied (Fig. 3). Incubation with 5 or 10 µg/ml 4C3 for up to 60 min failed to affect $P_{\text{alb}}$ (data not shown). Average $P_{\text{alb}}$ after incubation with 4C3 was $0.59 \pm 0.08$ ($n = 7$, $P < 0.01$ vs. control). These values represent calculations using average observed glomerular volume increase of 3.4% after 4C3 incubation and 8.4% for control glomeruli. P8E7 was not significantly increased by P8E7 ($0.12 \pm 0.25$, $n = 4$) or by a mAb to podocalyxin (BB5, $0.13 \pm 0.15$, $n = 9$) or laminin (SF7, $-0.39 \pm 0.14$, $n = 3$).

Incubation of 4C3 with rabbit PTPRO ECD fusion protein before glomerular incubation blocked binding of PTPRO to glomeruli and ameliorated the increase in $P_{\text{alb}}$ ($0.26 \pm 0.06$, $n = 4$), as shown in Fig. 3. In contrast, incubation of 4C3 with rabbit podocalyxin fusion protein did not inhibit glomerular binding and did not inhibit the increase in $P_{\text{alb}}$ ($0.66 \pm 0.16$, $n = 2$, data not shown).

Effect of polyclonal antibodies and mAbs directed against the ECD of PTPRO on $P_{\text{alb}}$ of isolated rat glomeruli. Anti-rat PTPRO polyclonal antibody bound to rat glomeruli and increased $P_{\text{alb}}$ ($0.56 \pm 0.05$, $n = 2$), as shown in Fig. 4, while BB5 and 4C3, which did not bind to rat glomeruli, did not increase $P_{\text{alb}}$ ($-0.07 \pm 0.10$, $n = 4$ and $-0.15 \pm 0.06$, $n = 4$, respectively, data not shown). Incubation of anti-rat PTPRO IgG with rat PTPRO ECD GST fusion protein prevented both glomerular binding and increase in $P_{\text{alb}}$ ($-0.20 \pm 0.20$, $n = 4$) (Fig. 4). Rat mAbs 1B4 and 1D1 each increased $P_{\text{alb}}$ ($0.54 \pm 0.15$, $n = 3$, and $0.44 \pm 0.27$, $n = 3$, respectively, $P < 0.01$), while control protein L11–135 did not affect $P_{\text{alb}}$ ($-0.08 \pm 0.12$, $n = 3$), as seen in Fig. 4. Preimmune rabbit serum had a small but statistically significant effect on $P_{\text{alb}}$ ($0.28 \pm 0.11$, $n = 11$, $P < 0.05$ vs. control). This effect was not concentration dependent and was comparable in magnitude to the changes induced by incubation with complement-containing serum in prior studies of complement-mediated injury (19).

To verify that altered volumetric responses were the result of altered permeability rather than changes in exchangeable volume or in capillary compliance, we performed additional studies of rat glomeruli treated for 60 min with polyclonal anti-rat PTPRO Ab. In these studies, the volumetric response to replacing medium containing BSA with that containing isoncotic HMW dextran (MW 200) was assessed using established protocols (18). Results are expressed as means ± SD for five glomeruli. Control glomeruli showed no change in volume when 4% BSA medium was replaced by dextran solution ($\Delta V = -0.03 \pm 0.45$). This finding confirms that $\sigma_{\text{alb}}$ and $\sigma_{\text{dextran}}$ did not differ. In contrast, antibody-treated glomeruli showed a decrease in volume ($\Delta V = -2.9 \pm 1.6\%$). This volume decrease confirms that anti-rat PTPRO decreased the control mAb BB5 did not bind to rabbit kidney cortex sections. Rat mAb to PTPRO ECD bound to rat sections; binding was inhibited by rat ECD fusion protein. These results are shown in Fig. 1.
ratio of $\sigma_{\text{alb}}$ to $\sigma_{\text{dextran}}$. Subsequent replacement of 4% dextran medium with 1% dextran led to comparable volume increase in control and experimental glomeruli ($\Delta V = 4.6 \pm 1.2$ and $5.9 \pm 0.8\%$, respectively). The equivalent glomerular volume responses of control and treated glomeruli to HMW dextran gradients is evidence that glomerular physical characteristics and $\sigma_{\text{dextran}}$ are not altered by antibody treatment. Taken together, these results provide strong evidence of decreased $\sigma_{\text{alb}}$ and increased $P_{\text{alb}}$ after treatment with anti-PTPro. Results are shown in Fig. 5.

**DISCUSSION**

We have demonstrated that antibodies to the ECD of PTPro increase $P_{\text{alb}}$ of isolated glomeruli, an effect seen with mAbs and polyclonal antibodies and in both rabbit and rat glomeruli. Effective antibodies to PTPro included mAb 4C3, which interacts with the fibronectin *domain 3* of rabbit ECD and decreases phosphatase activity of PTPro, and antibodies to rat ECD, including 1B4 and 1D1. Irrelevant mAbs and mAbs that lacked glomerular binding had no effect on $P_{\text{alb}}$. Fusion proteins composed of species-specific ECD interacted with the relevant antibodies and prevented glomerular binding as well as the increase in $P_{\text{alb}}$. These findings support the interpretation that the effects of antibodies on the permeability barrier are specific consequences of antibody interaction with the PTPro ECD.

In contrast to the effects of the other mAb and polyclonal Abs, mAb P8E7 had no effect on $P_{\text{alb}}$. P8E7, like 4C3, bound to glomeruli in a podocyte pattern and recognized PTPro protein on Western blotting under nonreducing conditions (25). However, the two mAbs differed in their specific binding to PTPro and in their effect on its phosphatase activity. Each recognized PTPro under nonreducing conditions, but under reducing conditions 4C3 antibody recognized a 205-kDa region representing PTPro while P8E7 did not. 4C3 binding was blocked by a fusion protein containing PTPro fibronectin repeat 3 while P8E7 binding was not blocked by this fusion protein (25). PTPro phosphatase activity was diminished by 4C3 compared with that seen with P8E7. Taken together, these results suggest that P8E7 recognizes a conformational epitope that is destroyed by denaturation and that fibronectin *domain 3* may participate in homophilic PTPro interactions. In addition, the association between diminished phosphatase activity and increased $P_{\text{alb}}$ suggests that tonic PTPro activity is required to maintain the normal characteristics of the glomerular filtration barrier.

The measurement of $P_{\text{alb}}$ in vitro was first described in studies of permeability induced by incubation with the polycation protamine, and the relevant principles are described in detail in that report (18). Since then, $P_{\text{alb}}$ has been measured in a wide variety of experimental models of glomerular injury (reviewed in Ref. 20). Under the standard conditions that we
have used, calculation of $P_{\text{alb}}$ is based on observations of glomerular volumetric responses to albumin oncotic gradients. The rationale for $P_{\text{alb}}$ measurements was based on prior observations that the glomerular capillary volume of intact isolated glomeruli varied directly with the oncotic composition of the medium in which they were suspended. Changes in glomerular volume with changes in bathing medium result from capillary expansion or collapse and is directly proportional to the effective oncotic gradient ($\sigma$). The oncotic gradient, in turn, represents the product of the chemical oncotic gradient, $\Delta I$, and the reflection coefficient, $\sigma$, of the solute employed. In normal

### Table 1

<table>
<thead>
<tr>
<th>Antibody</th>
<th>OD (410 nm)</th>
<th>PTPase Activity (ng PTPPro)</th>
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<tbody>
<tr>
<td>BB5 (control)</td>
<td>0.027</td>
<td>0</td>
</tr>
<tr>
<td>4B3 (podocalyxin)</td>
<td>0.018</td>
<td>-9</td>
</tr>
<tr>
<td>P8E7 (PTPro)</td>
<td>0.524</td>
<td>497</td>
</tr>
<tr>
<td>4C3 (PTPro)</td>
<td>0.273</td>
<td>246</td>
</tr>
</tbody>
</table>

**Fig. 2.** mAb 4C3 decreases phosphatase activity. **Top:** Western blotting was performed on glomerular protein using mAbs to PTPro (P8E7 and 4C3), podocalyxin (4B3), and control (BB5). The anti-PTPro Abs precipitated comparable amounts of PTPro, as shown by the arrows. The blot on the left was developed with 4C3 to identify PTPro. The blot on the right was developed with BB5. The bottom molecular weight bands are seen on both the left and right and show the heavy chains of the mAbs used for immunoprecipitation. **Bottom:** phosphatase activity of the immunoprecipitates was measured using the p-nitrophenylphosphate assay. Also shown are units of phosphatase activity developed from a standard curve made using recombinant rabbit PTPro intracellular domain. The 4C3 immunoprecipitate contains approximately half the phosphatase activity of the P8E7 immunoprecipitate.

**Fig. 3.** Rabbit mAb to PTPro increases albumin permeability ($P_{\text{alb}}$) of isolated glomeruli, an effect blocked by fusion protein. Glomeruli were isolated from normal rats and incubated with a polyclonal antibody to PTPro (PolyAb) with or without preincubation with a glutathione-S-transferase (GST) fusion protein of rat PTPro ECD (Poly+F). Other glomeruli were incubated with 1B4 or 1D1, mAbs to rat PTPro. Each of the three antibodies to PTPro caused a marked increase in $P_{\text{alb}}$. Preincubation with fusion protein prevented the increased $P_{\text{alb}}$ caused by the polyclonal antibody to PTPro. Incubation with the irrelevant mAb L11–132 (control) did not affect $P_{\text{alb}}$.

**Fig. 4.** Antibodies to PTPro increase $P_{\text{alb}}$ of isolated glomeruli, an effect blocked by fusion protein. Glomeruli were isolated from normal rats and incubated with a polyclonal antibody to PTPro (PolyAb) with or without preincubation with a glutathione-S-transferase (GST) fusion protein of rat PTPro ECD (Poly+F). Other glomeruli were incubated with 1B4 or 1D1, mAbs to rat PTPro. Each of the three antibodies to PTPro caused a marked increase in $P_{\text{alb}}$. Preincubation with fusion protein prevented the increased $P_{\text{alb}}$ caused by the polyclonal antibody to PTPro. Incubation with the irrelevant mAb L11–132 (control) did not affect $P_{\text{alb}}$.

**Fig. 5.** Antibodies to PTPro cause decrease in albumin reflection coefficient ($\sigma_{\text{alb}}$) but not $\sigma_{\text{dextran}}$. Volume change ($\Delta V$) of glomeruli incubated with an irrelevant antibody (control, black bars) or a polyclonal antibody to PTPro (open bars) was measured following medium change. In A and B, the change in oncotic pressure ($\Delta I$) was 12 mmHg. In C, there was no change in oncotic pressure. $A$: glomeruli were isolated in isoncotic medium containing 4% BSA, and medium was replaced with one containing 1% BSA. $\Delta V$ of glomeruli incubated with antibody to PTPro was significantly lower than control, indicating decreased $\sigma_{\text{alb}}$ and increased glomerular protein permeability. $B$: glomeruli were isolated in isoncotic medium containing high molecular weight (HMW) dextran, and medium was replaced with one containing HMW dextran of lower oncotic pressure. There was no significant difference in $\Delta V$ between control and experimental glomeruli. This is consistent with unchanged relative capillary volume, compliance, and $\sigma_{\text{dextran}}$. $C$: glomeruli were isolated in isoncotic medium containing 4% BSA, and medium was replaced by isoncotic medium containing HMW dextran. Control glomeruli did not change in volume when 4% BSA medium was replaced by isoncotic medium of HMW dextran, while experimental glomeruli showed significant capillary collapse and volume decrease.
glomeruli $\sigma_{\text{alb}}$ is $\sim1$. With injury to the filtration barrier, decreased $\sigma_{\text{alb}}$ decreases the effective oncotic gradient, diminishes capillary expansion, and results in a smaller steady-state capillary and total glomerular volume.

The finding that $P_{\text{alb}}$ is increased by an antibody that inhibits PTPro phosphatase while PTPro knockout animals fail to manifest proteinuria is in agreement with our findings in several models of renal disease (1, 7, 15, 16, 19). We have found that increased $P_{\text{alb}}$ is more sensitive and permits earlier identification of glomerular injury than does increased urinary protein in studies of antibodies to anti-Fx1A (19), of antibodies to $\beta1$-integrin (1), and of Dahl salt-sensitive rats (7) and of rats transgenic for the RF-2 gene of fawn-hooded hypertensive rats (16). Recent studies in PAN nephrosis also show increased $P_{\text{alb}}$ before detectable proteinuria (15). The current findings of increased $P_{\text{alb}}$ during experimental treatment are consistent with the concept that increased $P_{\text{alb}}$ provides a sensitive and early measurement of glomerular dysfunction.

PTPro has a large extracellular domain containing eight fibronectin type III-like repeats, a hydrophobic transmembrane segment, and a single PTPase domain (10, 25, 29). The knockout of PTPro caused changes in morphology of primary podocyte processes and decreased the total length of interdigitating tertiary processes and of the slit-diaphragm between them (30). PTPro (−/−) mice exhibit hypertension and elevated serum creatinine only after uninephrectomy or subtotal nephrectomy. These findings suggest that PTPro plays a role in the regulation of foot processes. They are, however, in apparent contradiction to the current findings that an antibody-induced decrease in phosphatase activity increases $P_{\text{alb}}$. It is possible that the glomeruli of PTPro (−/−) mice have abnormal $P_{\text{alb}}$ without proteinuria, a finding that could result from tubular reabsorption of filtered protein; this would be analogous to our findings in other models. Alternatively, other phosphatases may serve a redundant compensatory function in maintaining the protein permeability barrier when PTPro is absent throughout glomerular development. The increased expression of podocyte vimentin (30) is an example of changes in other glomerular proteins in PTPro (−/−) mice. This reasoning is supported by the finding that suppression of individual protein tyrosine phosphatase receptors does not necessarily lead to profound abnormalities in neuronal development (27).

Several members of type III receptor protein tyrosine phosphatases may serve cooperative as well as competitive functions. Similarly, multiple knockouts may be required to fully define the role of PTPro in podocyte differentiation and in maintenance of the permeability barrier.

Recent studies suggest that the extracellular domain of PTPro is homophilic (3, 24). Its signaling functions are incompletely understood and may be complex. For example, PTPro controls the sensitivity of ephrin receptors in retinal cells and determines their topographical growth (22). In these studies, PTPro dephosphorylates a phosphotyrosine residue conserved in the juxtamembrane region of ephrin receptors, which is required for the activation and signal transmission by these receptors. PTPro is also expressed in hematopoietic stem cells and plays a role in stem cell adhesion and in homophilic cell–cell interactions (2). It controls C B cell-receptor signaling and modulates tonic B cell receptor signaling (6).

A potential substrate for PTPro in neurons, neuronal pentraxin with chromo domain (NPCD), has been identified. This protein associates with PTPro in neurons and interacts with and may serve as a substrate for PTPro kinase activity (4, 5). NPCD is also expressed in glomeruli and colocalizes with PTPro. Its function in determining the outgrowth of cytoplasmic extensions from neurons may be analogous to its function in determining the normal morphology of podocyte foot processes. At present, the identity of the normal substrate for PTPro catalytic activity in glomeruli is not known, and the means by which this activity serves to maintain the filtration barrier in mature glomeruli remains to be determined.

Our results confirm that antibodies directed to PTPro cause increased glomerular protein permeability. In particular, antibodies to a specific region of the extracellular domain result in impairment of the permeability barrier. The possible role of phosphatase activity of PTPro in maintaining the glomerular protein permeability barrier is intriguing and worthy of further study. Results of the present studies support our hypothesis that tonic activity of PTPro is required to maintain the integrity of the filtration barrier. The study of isolated glomeruli provides the opportunity to define the effects of alterations in individual glomerular receptors and their signaling function on the filtration barrier. Our results emphasize the complexity of glomerular function and the potential role of altered tyrosine phosphorylation in its control.

ACKNOWLEDGMENTS

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GRANTS

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