Suppression of nephrin expression by TNF-α via interfering with the cAMP-retinoic acid receptor pathway

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Saito Y, Okamura M, Nakajima S, Hayakawa K, Huang T, Yao J, Kitamura M. Suppression of nephrin expression by TNF-α via interfering with the cAMP-retinoic acid receptor pathway. Am J Physiol Renal Physiol 298: F1436–F1444, 2010. First published March 17, 2010; doi:10.1152/ajprenal.00512.2009.—Nephrin, a crucial component of the slit diaphragm, is downregulated in proteinuric glomerular diseases including glomerulonephritis (9, 10, 15). Previous reports showed that TNF-α expression of nephrin in culture is regulated by the cAMP-RAR pathway and the suppressive effect of TNF-α on RAR was reversed by cAMP-elevating agents. These results suggest that 1) expression of nephrin in podocytes is regulated by the cAMP-RAR pathway and 2) suppression of nephrin by TNF-α is caused, at least in part, through selective inhibition of this pathway.

Nephrin, a podocyte-specific protein, is a key regulator that maintains normal structure and function of the slit diaphragm in the glomerulus (26). Loss of nephrin fails to form functional complexes in the slit membrane, resulting in dysfunction of the filtration barrier. In Finnish-type congenital nephrotic syndrome, massive proteinuria is caused by mutation of the nephrin gene (14). Similarly, disruption of the nephrin gene in mice results in abnormal foot process formation in podocytes and consequent nephrosis (21, 22). Previous reports showed that the level of nephrin decreases in several proteinuric glomerular diseases including glomerulonephritis (9, 10, 15). However, information is limited regarding mechanisms underlying the downregulation of nephrin under pathological conditions.

Cultured podocytes easily lose expression of nephrin in vitro. We previously reported (31, 38) that physiological ligands of nuclear receptors including all-trans-retinoic acid (ATRA) and 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) allow podocytes to maintain expression of nephrin in culture at both mRNA and protein levels. We also identified recently (19) that the nephrin gene promoter is activated by ATRA and 1,25(OH)2D3, which is mediated by the retinoic acid (RA) receptor (RAR) and the vitamin D receptor (VDR) but not the retinoid X receptor (RXR). On the other hand, activity of the nephrin gene promoter is downregulated by by-stander macromolecules as well as macrophage-derived cytokines including tumor necrosis factor-α (TNF-α) and IL-1β. This repression is reversible and observed in both basal and ATRA/1,25(OH)2D3-inducible nephrin expression (30). However, currently it is unknown whether and how inflammatory cytokines affect the function of RAR and VDR.

ATRA is an active metabolite of vitamin A and a ligand of RAR. After binding of ATRA to RAR, RAR forms homodimers or heterodimers with RXR, and the resultant complexes exert biological effects via binding to the retinoic acid response element (RARE) (37). Three putative RAREs are present in the regulatory region of the human nephrin gene (28). Similarly, 1,25(OH)2D3 is a biologically active metabolite of vitamin D that acts via its nuclear receptor, VDR. After binding of 1,25(OH)2D3 to VDR, the resultant complexes exert effects through the vitamin D response element (VDRE) (5). Using database analyses, we found three putative VDREs in the 1,25(OH)2D3-responsive 5.4-kb promoter of the murine nephrin gene (38).

In general, TNF-α activates several signaling pathways such as nuclear factor-κB (NF-κB) and mitogen-activated protein (MAP) kinases including extracellular signal-regulated kinase (ERK), p38 MAP kinase, and c-Jun NH2-terminal kinase (JNK) (2). The phosphatidylinositol 3-kinase (PI3K)-Akt pathway and the cAMP-protein kinase A (PKA) pathway may also be activated by TNF-α (17, 30, 34). In the present study, we first investigated effects of TNF-α on the activity of RAR and VDR, using reporter podocytes. We next examined involvement of downstream targets of TNF-α in the regulation of RAR and VDR, by interfering with the cAMP-RAR pathway independently of NF-κB, MAP kinases, and the PI3K-Akt pathway.

MATERIALS AND METHODS

Reagents. The following reagents were used: TNF-α (human recombinant, 10–20 ng/ml; Genzyme, Cambridge, MA), 1,25(OH)2D3 (1–10 nM; Chugai Pharmaceutical, Tokyo, Japan), ZK159222 (VDR antagonist, 1 μM; a gift from Dr. Seiichi Ishizuka, Teijin Pharma,
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Tokyo, Japan). ATRA (1–100 nM; Genzyme), LE540 (RAR pan-antagonist, 3 μM; a gift from Dr. Hiroyuki Kagechika, Tokyo Medical and Dental University, Tokyo, Japan), SC-514 (IKK2/NF-κB inhibitor, 50 μg/ml; Biomol International, Plymouth Meeting, PA), DHMEQ (NF-κB inhibitor, 5 μg/ml; a gift from Dr. Kazuo Umezawa, Keio University, Tokyo, Japan) (1), SP-600125 (JNK inhibitor, 25 μM; Sigma-Aldrich Japan, Tokyo, Japan), JNK inhibitor III (10 μM; Calbiochem, San Diego, CA), SB-203580 (p38 MAP kinase inhibitor, 25 μM; Sigma-Aldrich Japan), Akt-i/2 (Akt inhibitor, 10 μM; Calbiochem), H-89 (PKA inhibitor, 10–20 μM; Sigma-Aldrich Japan), forskolin (cAMP-elevating agent, 10 μM; Sigma-Aldrich Japan), and 3-isobutyl-1-methylxanthine (IBMX) (phosphodiesterase inhibitor, 250 μM; Sigma-Aldrich Japan).

Cell culture. Murine podocytes (24) were kindly provided by Dr. Karlhans Endlich (University of Heidelberg, Heidelberg, Germany) and cultured as described previously (30, 38). For the maintenance and/or propagation, podocytes were cultured on type I collagen-coated plates with Dulbecco’s modified Eagle’s medium–Ham’s F-12 and supplemented with 10% fetal bovine serum (FBS). Assays were performed in the presence of 1% FBS.

Establishment of stable transfectants. Nephrin reporter podocytes (MP/nephrin-SEAP) were established by stable transfection of murine podocytes with pN5.4-SEAP, which introduces the secreted alkaline phosphatase (SEAP) gene under the control of the 5.4-kb murine nephrin gene promoter (30). Reporter podocytes to evaluate activity of RAR (MP/RARE-SEAP) were established by stable transfection with pRARE-SEAP (BD Biosciences, Palo Alto, CA), which introduces SEAP under the control of two copies of RAREs. Reporter podocytes to evaluate activity of VDR (MP/VDRE-Luc) were established by stable transfection with pVDRE-Luc (kindly provided by Dr. David W. Russell, University of Texas Southwestern Medical Center, Dallas, TX), which intoduced luciferase under the control of three copies of the VDRE consensus sequence (originally designated as pSPPX3-TK-LUC) (3). Reporter podocytes to evaluate activity of the cAMP-PKA pathway (MP/cRE-SEAP) were established by stable transfection with pCRE-SEAP (BD Biosciences), which introduces SEAP under the control of three copies of the cAMP response element (CRE). MP/Neo-RARE-SEAP cells and MP/Neo-M-RARE-SEAP cells were established by stable cotransfection of podocytes with pRARE-SEAP and pcDNA3.1 (Invitrogen, Carlsbad, CA) or pIκBα/MSN, which introduces a dominant-negative mutant of IκBα (IκBαM; a gift from Dr. Inder M. Verma, Salk Institute, La Jolla, CA) (33), respectively. MP/JNK-NK-RARE-SEAP cells were established by stable cotransfection of podocytes with pRARE-SEAP and pcDNA-JNK1 (APF), which introduces a dominant-negative mutant of JNK (a gift from Dr. Roger J. Davis, University of Massachusetts Medical Center, Worcester, MA). Transfection was performed with GeneJuice Transfection Reagent (Novagen).

Transient transfection. With the use of GeneJuice, podocytes were transiently transfected with pCRE-SEAP or cotransfected with pIκBα/MSN and pNfκB-Luc (Panomics, Fremont, CA), which introduces a luciferase gene under the control of the IκBα sites. The cells were then stimulated with TNF-α and subjected to chemiluminescent assay.

Chemiluminescent assays. Activity of SEAP and luciferase was evaluated by chemiluminescent methods with the Great EscAPe SEAP Detection Kit (BD Biosciences) and the Luciferase Assay System (Promega, Madison, WI), respectively (13). The values were normalized by the number of viable cells estimated by formazan assay.

Formazan assay. The number of viable cells was assessed by a formazan assay with Cell Counting Kit-8 (Dojindo Laboratory, Kumamoto, Japan).

Western blot analysis. Western blot analysis of kinases was performed by the enhanced chemiluminescent system (Amersham Biosciences, Little Chalfont, UK) and PhosphoPlus p44/42 MAP Kinase (Thr202/Tyr204) Antibody Kit, PhosphoPlus SAPK/JNK (Thr183/Tyr185) Antibody Kit, PhosphoPlus p38 MAP Kinase (Thr180/Tyr182) Antibody Kit, and PhosphoPlus Akt (Ser473) Antibody Kit (Cell Signaling, Beverly, MA) according to protocols provided by the manufacturer. Western blot analysis of nephrin was performed with anti-nephrin antibody (N-20: sc-19000; Santa Cruz Biotechnology, Santa Cruz, CA), as described previously (38). As a loading control, the level of β-actin was evaluated with anti-β-actin antibody (Sigma-Aldrich Japan).

Northern blot analysis. Total RNA was extracted by a single-step method, and Northern blot analysis was performed as described previously (16). An IκBαM cDNA (33) was used to prepare a radiolabeled probe. Expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control.

Statistical analysis. Assays were performed with n = 4–8. Data are expressed as means ± SE. Statistical analysis was performed by using a nonparametric Mann-Whitney U-test to compare data in different groups. A P value <0.05 was considered to indicate a statistically significant difference.

RESULTS

Suppression of nephrin gene promoter by TNF-α via inhibition of RAR but not VDR. We previously reported (31) that expression of nephrin in cultured podocytes is upregulated by a differentiation medium, namely, vitamin D3- and RA-supplemented DMEM/F12 (VRAD). This effect is mediated by RAR and VDR (19). We also elucidated (30) that TNF-α suppresses expression of the nephrin gene in podocytes cultured in the presence of 1,25(OH)2D3 and ATRA. Using MP/nephrin-SEAP reporter cells cultured in VRAD, we first examined an effect of TNF-α on the activity of the nephrin promoter.
gene promoter. The cells were first incubated in VRAD and treated with TNF-α for 24 h. Chemiluminescent assay revealed that VRAD induced activation of the nephrin gene promoter and that this induction was significantly suppressed by TNF-α (Fig. 1A). The suppression of the nephrin gene promoter was correlated with downregulation of nephrin protein by TNF-α (Fig. 1B). To elucidate mechanisms underlying this observation, we first tested involvement of VDR. For this purpose, reporter podocytes were established to evaluate activity of VDR. The established MP/VDRE-Luc cells exhibited induction of luciferase in response to 1,25(OH)2D3 in a dose-dependent manner. This activation was abrogated in the presence of VDR antagonist ZK159222 (Fig. 2A). Of note, treatment with ZK159222 alone modestly activated VDRE. This is because ZK159222 possesses a weak agonistic activity for VDR (Dr. Seiichi Ishizuka, Teijin Pharma, personal communication). MP/VDRE-Luc cells were then incubated in the absence or presence of 1,25(OH)2D3 and exposed to TNF-α. As shown in Fig. 2B, activity of VDR was not affected by TNF-α under both 1,25(OH)2D3-stimulated and -unstimulated conditions. This result suggests that VDR is not a target for the suppressive effect of TNF-α on nephrin gene expression.

To investigate involvement of RAR, we next developed reporter podocytes for RAR. The established MP/RARE-SEAP cells exhibited a dose-dependent induction of SEAP activity in response to ATRA (Fig. 3A). The activation of RAR was abrogated in the presence of RAR pan-antagonist LE540 (Fig. 3B). MP/RARE-SEAP cells were then cultured in the absence or presence of ATRA and exposed to TNF-α. As shown in Fig. 3C, activity of RAR was significantly suppressed by TNF-α under both ATRA-stimulated and -unstimulated conditions. Consistent with these results, ATRA elevated the level of nephrin protein, which was suppressed by the treatment with TNF-α (Fig. 3, D and E). These results suggest that RAR is a target for the suppressive effect of TNF-α on nephrin expression.

As shown in Fig. 2B, activity of VDR was not affected by TNF-α under both 1,25(OH)2D3-stimulated and -unstimulated conditions. This result suggests that VDR is not a target for the suppressive effect of TNF-α on nephrin gene expression.

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Lack of involvement of NF-κB, MAP kinases, and Akt in suppression of RAR by TNF-α. In general, TNF-α activates several signaling pathways that involve NF-κB, MAP kinases, and Akt (2, 30, 34). To disclose molecular events involved in the suppression of RAR by TNF-α, roles of individual pathways were examined. To confirm activation of NF-κB by TNF-α, podocytes were transiently transfected with pNFκB-Luc, stimulated by TNF-α, and subjected to luciferase assay. As shown in Fig. 4A, TNF-α induced significant activation of NF-κB in podocytes. To examine a role of NF-κB inhibition in the suppressive effect of TNF-α, MP/RARE-SEAP cells cultured with or without ATRA were exposed to TNF-α in the absence or presence of NF-κB inhibitor SC-514. As shown in Fig. 4B, center, suppression of RAR by TNF-α was not attenuated in the presence of SC-514. A similar result was also obtained with another NF-κB inhibitor, DHMEQ (Fig. 4B, right). Of note, the results were confirmed by additional experiments (Fig. 4C).
the concentrations of SC-514 and DHMEQ are sufficient to inhibit TNF-α-induced NF-κB activation or NF-κB-dependent expression of monocyte chemotactic protein 1 (data not shown). Consistent with these results, overexpression of a superrepressor mutant of IkBα, IkBaM, abrogated activation of NF-κB by TNF-α (Supplemental Figs. S1 and S2), whereas it did not reverse the suppressive effect of TNF-α on RAR (Fig. 4C).1 These results suggest lack of involvement of NF-κB in the suppression of RAR by TNF-α.

We next examined roles of MAP kinases. When podocytes were treated with TNF-α, phosphorylation of JNK and p38 MAP kinase was rapidly induced, whereas phosphorylation of ERK was not evident (Fig. 5A). To examine roles of JNK and p38 MAP kinase in the suppression of RAR by TNF-α, MP/RARE-SEAP cells were exposed to TNF-α in the presence of their selective inhibitors, SP-600125 and SB-203580. Even in the presence of these inhibitors, suppression of TNF-α on RAR was similarly observed (Fig. 5B). Consistent with these results, pharmacological inhibition of JNK by JNK inhibitor III or dominant-negative inhibition of JNK with MP/JNK-RARE-SEAP cells did not reverse the suppressive effect of TNF-α on RAR (data not shown). These results suggest that suppression of RAR by TNF-α is independent of MAP kinases.

Previous reports suggested that the PI3K-Akt pathway may be activated in response to TNF-α (34). To examine a role of Akt in the suppression of RAR by TNF-α, we first tested whether or not TNF-α induces activation of Akt in podocytes. Podocytes were treated with TNF-α for up to 24 h in the absence or presence of ATRA, and phosphorylation of Akt was evaluated. As shown in Fig. 6A, Akt was phosphorylated by TNF-α, and both basal and inducible phosphorylation of Akt was not influenced by ATRA. We further examined involvement of Akt in the suppressive effect of TNF-α on RAR. MP/RARE-SEAP cells cultured with or without ATRA were exposed to TNF-α in the absence or presence of a selective inhibitor of Akt1/2, Akti-1/2. Even in the presence of Akti-1/2, suppression of TNF-α on RAR was similarly observed (Fig. 6B), suggesting lack of involvement of Akt.

Involvement of cAMP-PKA pathway in suppression of RAR by TNF-α. In contrast to the well-known stimulatory effects of TNF-α on the NF-κB, MAP kinase, and Akt pathways, little is known about influences of TNF-α on the cAMP-PKA pathway. However, a recent report indicated that TNF-α may affect the cAMP-PKA signaling in synovial fibroblasts (17). Furthermore, another report suggested that phosphorylation of PKA potentiated activity of RAR in human mammary cancer cells (11). We hypothesized that, in podocytes, TNF-α could down-regulate the cAMP-PKA pathway and thereby attenuate activity of RAR. To examine this possibility, podocytes were transiently transfected with pCRE-SEAP, and basal activity of the cAMP-PKA pathway was assessed. Chemiluminescent assay revealed that under the basal culture condition podocytes exhibited substantial CRE activity, and it was abolished by the treatment with the PKA inhibitor H-89 (Fig. 7A). When MP/ATRA was added, the CRE activity was further reduced (Fig. 7B). These results suggest lack of involvement of Akt in the suppression of RAR by TNF-α.

1 The online version of this article contains supplemental material.
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RARE-SEAP cells were treated with cAMP-elevating agent forskolin or IBMX, significant activation of RAR was observed (Fig. 7B). On the other hand, the PKA inhibitor H-89 significantly suppressed activity of RAR under both unstimulated and ATRA-stimulated conditions (Fig. 7C). Furthermore, suppression of RAR by TNF-α was reversed by the treatment with forskolin (Fig. 7D).

A previous report suggested that RA may increase cAMP levels in podocytes (12). To examine effects of ATRA and TNF-α on the level of cAMP, we established reporter podocytes for CRE. Consistent with the result of transient transfection, the established MP/CRE-SEAP cells exhibited substantial basal activity of the cAMP pathway, and it was further enhanced by the treatment with forskolin (Fig. 7E). With this reporter cell, effects of ATRA and TNF-α on the activity of cAMP were investigated. In our experimental setting, ATRA did not induce significant activation of CRE (Fig. 7F). However, treatment with TNF-α significantly inhibited basal activity of CRE (Fig. 7G). These results suggest that, in podocytes, TNF-α downregulates basal activity of the cAMP-PKA pathway that is essential for activation of RAR and consequent nephrin gene expression.

To further confirm our conclusion, podocytes were treated with H-89 or forskolin and subjected to Western blot analysis of nephrin. Consistent with the result shown in Fig. 7C, suppression of PKA by H-89 downregulated the level of nephrin protein (Fig. 8A). On the other hand, forskolin, the activator of the cAMP-PKA pathway, enhanced the basal level of nephrin protein, as shown in Fig. 8B.

DISCUSSION

Expression of nephrin in podocytes is preserved by RA and 1,25(OH)2D3 (31, 38). This effect is mediated by RAR and VDR (19). On the other hand, expression of nephrin in podocytes is downregulated by TNF-α (30). In the present investigation, we found that, in podocytes, TNF-α selectively represses activity of RAR but not VDR. In this process, well-known downstream targets of TNF-α including NF-κB, MAP kinases, and Akt are not involved. We identified that TNF-α depresses basal activity of the cAMP-PKA pathway that is essential for the maintenance of RAR activity. Our present results revealed for the first time that 1) expression of nephrin in podocytes is maintained by the cAMP-RAR pathway and 2) suppression of nephrin by TNF-α is caused, at least in part, through selective inhibition of this pathway. An outline of our present hypothesis is illustrated in Fig. 9.

In the present study, we demonstrated that activity of the cAMP pathway was suppressed by TNF-α. Molecular mechanisms underlying this observation are currently unclear. Patrizio (20) reported that treatment of microglia with TNF-α suppressed accumulation of cAMP triggered by forskolin. The author also showed that, in the cAMP pathway, TNF-α acted at the step of adenyl cyclase but not the step of G proteins or phosphodiesterases. TNF-α reduced the ability of adenyl cyclase by 50% under both basal and inducible situations (20). Another previous report showed that generation of nitric oxide (NO) via inducible nitric oxide synthase (iNOS) inhibited forskolin-stimulated cAMP production by adenyl cyclase and cAMP-dependent ion transport in cholangiocytes (27). Because podocytes express iNOS in response to TNF-α (29), induction of iNOS, generation of NO, and consequent suppression of adenyl cyclase may be involved in the TNF-α-induced downregulation of the cAMP-RAR pathway. However, although Patrizio indicated involvement of NF-κB in the suppressive effect of TNF-α, our present results showed that NF-κB does not contribute to the suppression of the cAMP-RAR pathway by TNF-α. Other downstream targets of TNF-α including MAP kinases and Akt were also uninvolved. In light of the current knowledge that induction of iNOS by TNF-α is NF-κB dependent (36), other mechanisms should be involved in the suppressive effect of TNF-α on cAMP. Further investigation will be required to disclose molecular events underlying the effect of TNF-α.

In podocytes, TNF-α downregulated the cAMP-RAR pathway. However, effects of TNF-α on cAMP and RAR are a little complicated. Patrizio (20) reported that although the suppression of cAMP by TNF-α was observed in microglia, it was not detectable in astrocytes. Another previous report showed that TNF-α, as well as IL-1β and IL-6, caused a shift of RARα and RARβ from the cytosolic compartments into the nuclei in Schwann cells. These cytokines also enhanced nuclear translocation of RARs by ATRA in the same cell type (18). In contrast, we showed in the present study that in podocytes activity of the cAMP-RAR pathway was inhibited by TNF-α. The effect of TNF-α on cAMP-RAR is, supposedly, different.
from cell type to cell type, and the suppressive effect may be relatively specific to particular cell types including podocytes.

In this report, we showed that TNF-α did not affect activity of VDR. In contrast to our present result, a previous report indicated that TNF-α inhibited 1,25(OH)2D3-induced activation of VDR in CV-1 cells (8). This was associated with decreased binding of VDR to VDRE from the osteocalcin gene. In contrast, in podocytes, basal and 1,25(OH)2D3-triggered activation of VDR was not affected by TNF-α. The reason for the discrepancy is currently unclear, but regulation

Fig. 7. Involvement of the cAMP-protein kinase A (PKA) pathway in the activation of RAR and its suppression by TNF-α. A: podocytes transiently transfected with pCAMP response element (CRE)-SEAP were treated with 10 μM H-89 for 24 h and subjected to chemiluminescent assay. B: MP/RARE-SEAP cells were treated with ATRA, 10 μM forskolin, or 250 μM 3-isobutyl-1-methylxanthine (IBMX) for 24 h and subjected to analysis. C: MP/RARE-SEAP cells were treated with H-89 in the absence or presence of ATRA and subjected to analysis. D: MP/RARE-SEAP cells were treated with TNF-α in the absence or presence of ATRA and/or forskolin and subjected to analysis. E–G: MP/CRE-SEAP cells were treated with forskolin (E), ATRA (F), or TNF-α (G) for 24 h and subjected to analysis. *P < 0.05.

Fig. 8. Regulation of nephrin protein by the cAMP-PKA pathway. Podocytes were treated with 7.5 μM H-89 (A) or 10 μM forskolin (B) and subjected to Western blot analysis of nephrin.

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of VDR in podocytes could be different from that in other cell types. As a reporter plasmid, we used pVDRE-Luc that contains VDRE derived from the mouse osteopontin gene. Another possibility is that the regulation of VDRE in the osteocalcin promoter could be different from that in the promoter of the osteopontin gene.

Regarding the interaction between RAR and the cAMP pathway, a previous report showed that the NH2-terminal AF-1 domain of RARα is phosphorylated by the cyclin-dependent kinase (cdk7)/cyclin H complex of TFIIH and its COOH-terminal AF-2 domain by PKA (23). Another report also showed that phosphorylation by PKA potentiates RARα activity by increasing interaction with and phosphorylation by cdk7/cyclin H (11). A similar mechanism might be involved in the activation of RAR by the cAMP pathway in podocytes.

In normal kidneys, cAMP, but not cGMP, is present abundantly in podocytes (4). Consistent with this result, we elucidated that the cAMP pathway is constitutively active in cultured podocytes. Currently, the physiological meanings of this abundant cAMP in podocytes are unclear, but accumulating data indicate important cAMP in regulating podocyte morphology, actin assembly, matrix production, mitogenesis, hormonal responses, function of slit membrane-associated proteins, and glomerular permeability (7). In particular, increased cAMP levels in podocytes exert glomeruloprotective effects. For example, several investigators suggested that cAMP controls glomerular permeability (7). Studies on isolated glomeruli indicated that cAMP reduces glomerular permeability by a direct action on glomerular cells (25, 35). Furthermore, treatment of experimental glomerulonephritis with a phosphodiesterase inhibitor effectively reduced albuminuria (32). Podocytes control glomerular permeability via reorganization of the actin cytoskeleton, through changes in the contractile state of foot processes, or by direct phosphorylation of slit membrane-associated proteins, e.g., nephrin, CD2-associated protein, and ZO-1 (6). In addition to these putative mechanisms, our present results suggest another possibility underlying the renoprotective effect of cAMP. That is, cAMP may maintain nephrin expression in podocytes via the RAR pathway and thereby contribute to prevention of proteinuria.