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Alterations in cytoskeletal protein expression by mycophenolic acid in human mesangial cells requires Rac inactivation

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ABSTRACT

In response to glomerular injury, mesangial cells are activated into myofibroblasts, which contribute to the physiopathology of glomerulosclerosis. We have previously shown that chronic treatment of cultured human mesangial cells with mycophenolic acid (MPA), a specific inhibitor of guanosine nucleotide synthesis, prevents their activation and alters cytoskeleton protein expression and associated functions, such as contractility and migratory capacity. The aim of the present study was to explore the mechanisms underlying MPA-induced mesangial cytoskeleton alterations.

We therein show that coincubation with guanosine (100 μM) compensates for the effects of MPA on mesangial cell proliferation and migration, and prevents MPA-induced overexpression of alpha-smooth muscle actin (SMA) and basic calponin (b-calp), indicating that guanylates are involved in mesangial responses to MPA. MPA decreased the GTP-bound (active) form of both RhoA, Rac1 and Cdc42, and specifically altered the expression level of Rac1. Pharmacological inhibition of RhoA activity reduced expression of both SMA and calponin, whereas overexpression of a dominant-negative form of Rac1 increased SMA expression. Conversely, overexpression of constitutively active Rac1 resulted in SMA and b-calp down-regulation, and fully prevented their stimulation by MPA, indicating that Rac inactivation is responsible for MPA effects on mesangial cytoskeletal expression.

These results show that in human mesangial cells, RhoA and Rac1 exert opposite effects on the expression of two major cytoskeletal proteins: SMA and basic calponin. Moreover, these data highlight for the first time an integrated mechanism whereby MPA regulates mesangial phenotype, which is mediated by loss of Rac activity.

1. Introduction

Mycophenolic acid (MPA) is a selective noncompetitive inhibitor of inosine 5′-monophosphate dehydrogenase, the rate-limiting enzyme in the *de novo* synthesis of guanine ribo- and 2′-deoxyribonucleotides. MPA and its derivative mycophenolate mofetil (MMF) act as immunosuppressive drugs, which have been shown to be effective for the prevention and treatment of renal allograft rejection. The function of the transplanted kidney was markedly improved when MMF was added and

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calcineurin inhibitor doses were reduced [1,2] through reduction of immune- and non-immune-mediated renal damage. In animal studies, MMF ameliorated renal lesions in immune-mediated disease, such as the anti-thy 1.1 model or the obese diabetic Zucker rat [3,4], but was also effective in non-immune-mediated renal damage [5,6]. In humans, MMF have been used in small number of patients in a variety of renal diseases, and conclusions need to be confirmed in larger studies. Nevertheless, recent studies show that MMF significantly reduced proteinuria focal and segmental glomerulosclerosis [7].

These observations led us to study the direct effects of MPA on mesangial cells. Mesangial cells contain a cytoskeleton capable of contraction that is involved in the redistribution of glomerular capillary blood flow, and is disorganized in long-term glomerular diseases such as diabetic glomerulopathy [8]. We used an *in vitro* model of human mesangial cells transformed with the large T antigen of SV40 (the IP15 cell line) to demonstrate that, besides its antiproliferative effects, chronic MPA treatment decreases the migration and contractile activities of mesangial cells, an effect that is associated with alterations in cytoskeleton expression and dynamics [9]. In particular, MPA induces a strong overexpression of alpha-smooth muscle actin (SMA) in IP15 cells, an effect that was also observed in early-passage, non-transformed, human mesangial cells [10].

Based on the above findings, we hypothesized that MPA, by decreasing the intracellular level of guanine nucleotides, may alter the function of GTP-dependent proteins of the Rho/Rac family, which are known to control organization and dynamics of the actin cytoskeleton. Like most of the small GTP-binding proteins, Rho GTPases cycle between an inactive GDP-bound and an active GTP-bound form. The best characterized members of this family are RhoA, Rac1 and Cdc42, which regulate actin dynamics by acting as molecular switches that transduce signals from activated membrane receptors to cytoskeleton organizers. When micro-injected into fibroblasts, constitutively activated mutants of RhoA generate actin stress fibers, while those of Rac1 induce ruffles and lamellipodia at the cell periphery, and Cdc42 active mutants stimulate the formation of microspikes or filopodia [11]. Moreover, these proteins were also recently shown to regulate SMA expression in vascular smooth muscle cells [12].

Our results show that treatment with MPA actually decreased the percentage of activated forms of both GTPases; more surprisingly, MPA also decreased the expression level of Rac1. Then, we found that pharmacological inhibition of Rho decreased, while genetic inhibition of Rac1 increased the expression level of SMA in IP15 cells. Thus, Rac1 inhibition mimics MPA effect on mesangial SMA. Consistent with this, expression of constitutively activated Rac1 prevents MPA-induced SMA and basic calponin expression, suggesting that MPA affects mesangial actin cytoskeleton by inhibiting Rac1 function.

2. Materials and methods

2.1. Chemicals

All products used for cell culture were from InVitrogen (Cergy-Pontoise, France). The human mesangial IP15 cell line used in this study has been described elsewhere [10]. Cells were grown in RPMI 1640 medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 1 mmol/L Na pyruvate, non-essential amino-acids, 1 mmol/L Hepes buffer, and 10% fetal calf serum (FCS) (exceptions mentioned). MPA, guanosine, and monoclonal antibodies against human SMA (1A4 clone), vimentin (VIM-132 clone), basic calponin (hCP clone) and α-tubulin (B-5-1-2 clone) were from Sigma Chemical (St. Louis, MO). Anti-RhoA, -Rac1 and -Cdc42 antibodies were purchased respectively from Santa Cruz, Upstate Biotechnology and Transduction Laboratories. In most experiments, a 5-day treatment of IP15 cells with 1 μM MPA was performed to reach maximal intracellular GTP depletion (exceptions mentioned).

Recombinant TAT-C3 protein (*Clostridium botulinum* C3 exoenzyme linked to the C-terminal of the HIV TAT protein transduction domain) was a kind gift of Dr. Gervaise Loirand (INSERM U533, Nantes, France) [13].

2.2. Immunofluorescence microscopy

Subconfluent cells grown on glass coverslips were fixed with 3% paraformaldehyde and fluorescently labeled as previously described [9]. Images were obtained by using a Zeiss photomicroscope equipped with an Axiocam digital camera.

2.3. Western-blot analysis

Total proteins were extracted from mesangial cells and submitted to Western-blot analysis as described previously [10]. A 15% polyacrylamide gel was used for analysis of Rho or Rac protein expression, and 8–10% gels were used for cytoskeletal proteins. Total protein amount was quantitated using the Micro BC Assay (Interchim, Montucon, France) to ensure equal loading of lanes in comparative studies; this was confirmed either by vimentin labeling or after conterstaining the gel with Coomassie blue. Exposed films were numerized using a Sony digital camera and densitometric analysis was performed using the NIH Image 1.62 program for Macintosh. Quantitations were performed on at least two different Western-blots for each sample.

2.4. Cell proliferation and migration assays

Cell proliferation was estimated using crystal violet staining as previously described [14]. Absorbance at 570 nm was red after extraction by the 50% ethanol/1% acetic acid buffer, and was proportional to the number of cell nuclei present in the culture well.

Migration assay was performed by wounding a layer of confluent, growth-arrested cells as previously described [9]. Reduction in the wound area was quantitated using NIH Image 1.62 software (National Institutes of Health).

2.5. RhoA, Rac1 and Cdc42 activity assays

Active (GTP-bound) forms of RhoA, Rac1 or Cdc42 were captured via the pull-down assay, and quantitated by Western blotting of the precipitated material, as previously described [15]. Rho-GTP was captured using GST-Rho binding domain-rhotekin, and Rac-GTP and Cdc42-GTP were both

captured using GST-Cdc42/Rac-interactive binding domain (CRIB)-PAK.

2.6. Expression constructs and transfection of IP15 cells

Constructs encoding wild-type (Rac WT), constitutively active (Rac QL), or constitutively inactivated (Rac N17) mutants of Rac were made and kindly provided by Dr. Silvio Gutkind (NIH, Bethesda, MD, USA) [16]. All constructs contain a neomycin resistance gene. IP15 cells were transformed by electroporation, and stable transfectants were selected by culturing transfected cells in the presence of 0.15 mg/mL of Geneticin (InVitrogen), a dose that was selected from preliminary experiments on the basis of its ability to kill 100% of non-transformed IP15 cells within 7 days.

2.7. siRNA transfection

Small interference RNAs (siRNAs) sequences efficiently targeting against human Rac1 were as described in [17]. A scramble 21-mer siRNA was used as a control. Both siRNA were chemically synthesized as duplexes by Eurogentec (Searing, Belgium), and were introduced in IP15 cells using Lipofectamine (InVitrogen, Cergy Pontoise, France) at a concentration of 125 nM, according to supplier's instructions.

2.8. Statistical analysis

Statistical analyses on quantitative data were carried out by analysis of variance (ANOVA) and comparison of means was performed using Student's *t*-test (Statview®). For all experiments, p values <0.05 were considered as significant. Statistical analyses of Western-blot gels were performed using paired *t*-test, because of the semiquantitative nature of these results.

3. Results

3.1. Addition of guanosine prevented the effects of mycophenolic acid

As inosine-5′-monophosphate dehydrogenase inhibition is the primary effect of MPA, we first tested whether the effects of MPA on mesangial cells were solely dependent on guanidylic nucleotide depletion. Serum-induced cell proliferation was usually inhibited in a reversible manner by a 3-day treatment with 1 μM MPA, a dose that was not cytotoxic [10]. Cell proliferation activity was restored in a dose-dependent manner when guanosine was added simultaneously with MPA (Fig. 1A). The prevention of the anti-proliferative effect of MPA was complete at 100 μM guanosine, a concentration previously shown in other cell types to replete the guanine nucleotide pool via the salvage pathway [18]. Similarly, addition of 100 μM guanosine prevented the MPA-induced inhibition of cell migration (Fig. 1B) and the overexpression of smooth-muscle α-actin and basic calponin (Fig. 1C). Guanosine alone at 100 μM neither significantly affected mesangial cell proliferation or migration, nor changed the expression level of SMA and basic calponin (Fig. 1).

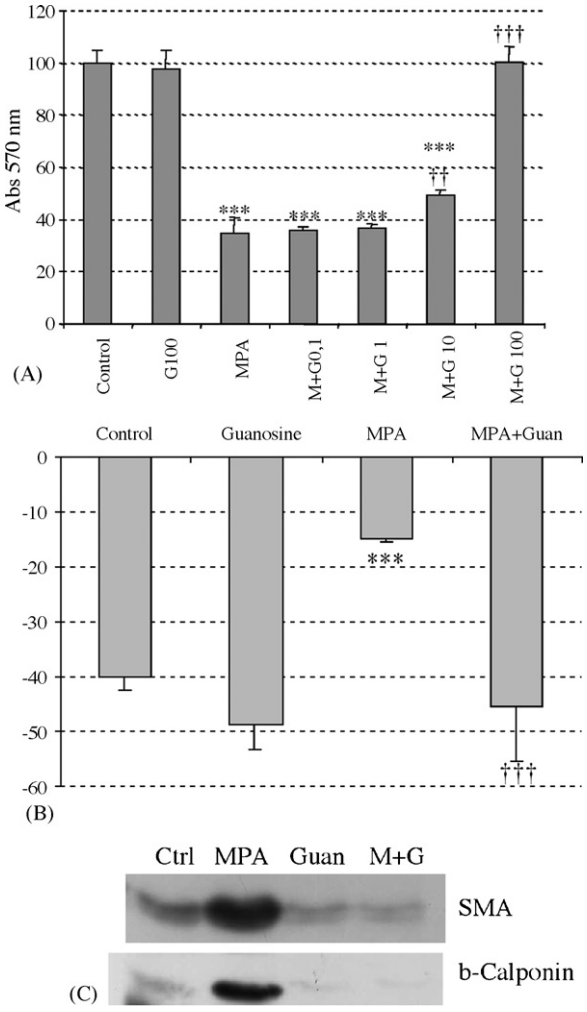

Fig. 1 – Co-incubation of IP15 cells with supraphysiological doses of guanosine prevents the effects of MPA. (A) IP15 cells were seeded at 10,000 cells/cm^2 in 24-well plates and treated for 3 days with or without MPA (1 μM) and/or guanosine (0.1–100 μM). At the end of the treatment period, the number of cells per well was estimated by measuring the absorbance at 570 nm after crystal violet labeling (see methods). Graph represents values related to control cells. Data are mean ± S.D. from at least eight experimental points per condition, and from four different cultures. (***) $p < 0.0001$ vs. control; (††) $p < 0.001$, (†††) $p < 0.0001$ vs. MPA-treated cells. (B) Migratory activity of IP15 cells. Confluent, growth-arrested cells were pretreated for 5 days with MPA (1 μM) and/or guanosine (100 μM), then wounds were made by scraping the cell layer using a sterile pipette tip. Results represent the percentage of reduction in wound width after 3 days. $p < 0.0001$ vs. control; (†††) $p < 0.0001$ vs. MPA-treated cells. (C) Representative Western-blot analysis of protein expression in IP15 cells treated for 5 days with or without MPA (1 μM) and/or guanosine (100 μM). The membrane was then incubated with antibodies against alpha-smooth actin (SMA, 45 kDa) and basic calponin (34 kDa). Equal loading was assessed by Coomassie blue staining of the gel (not shown).

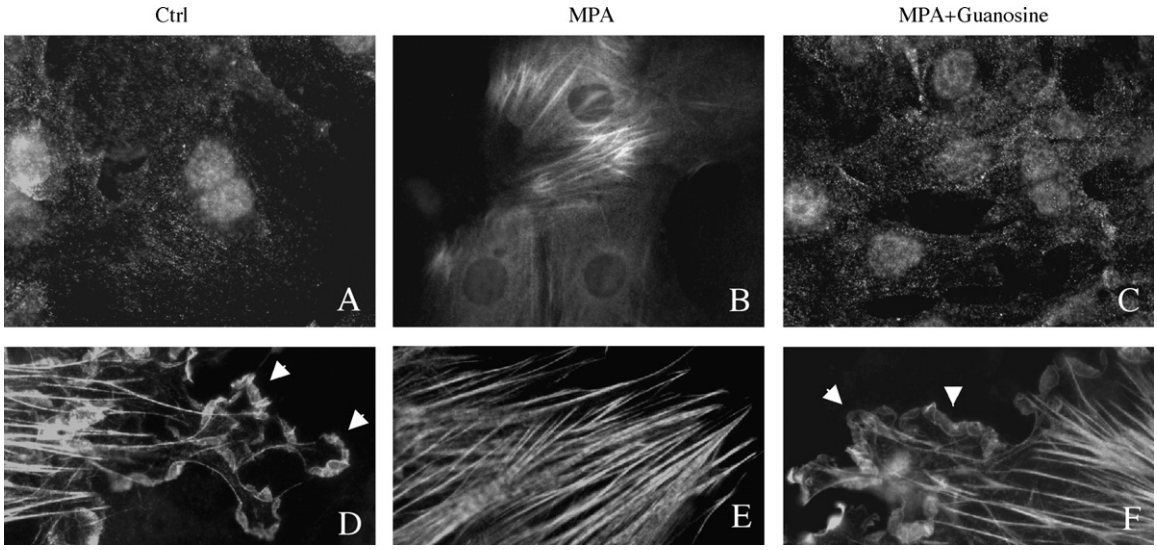

Fig. 2 – Guanosine prevents the effects of MPA on mesangial cytoskeleton organization. IP15 cells were incubated for 5 days in the absence (A and D) or presence of 1 μM MPA (B and E) or 1 μM MPA + 100 μM guanosine (C and F), and stained with antibodies specific for basic calponin (A–C, original magnification: 20× objective) or SMA (D–F, original magnification: 63× objective). Cytoskeletal localisation of calponin following actin fibers was induced by MPA (B), and prevented by co-incubation with guanosine (C). Membrane ruffling can be observed at the periphery of control cell labelled with SMA (D, arrows), and was inhibited by MPA treatment (E) but not in the presence of guanosine (F, arrows).

Immunochemistry also demonstrated that co-incubation with guanosine prevented the changes in the cytoskeleton organization induced by MPA. Non-confluent, untreated IP15 cells cultured in the presence of 10% serum exhibit numerous membrane ruffles labelled with anti-SMA antibody (Fig. 2D), providing evidence of an actin turnover at the cell periphery, which is necessary to the ability of cells to migrate [19]. Basic calponin staining show a preferential localisation in the nucleus (Fig. 2A). As previously described [9], MPA caused a relocalisation of calponin in the cytoplasm, where it was found associated with actin filaments (Fig. 2B), and prevented actin accumulation in membranes ruffles at the cell edges (Fig. 2E). Guanosine alone had no effect on the distribution of calponin and SMA (data not shown). Cells co-incubated with MPA and guanosin showed a cytoskeletal protein distribution similar to that found in untreated cells, where membrane ruffles remained abundant (Fig. 2C and F).

Taken together, these results indicate that inhibition of inosine monophosphate dehydrogenase and subsequent decrease in the intracellular guanosine nucleotide level is responsible for the observed effects of MPA on mesangial cell proliferation, migration and cytoskeletal alterations.

3.2. *GTPase activities are affected by mycophenolic acid treatment*

We hypothesized that the decrease in intracellular GTP level caused by MPA was responsible for a decrease in the activity of small GTP-binding proteins. We focused on the Rho/Rac family, which are important regulators of the actin cytoskeleton and cell motility. The amount of both RhoA, Rac1 and Cdc42 activated (GTP-bound) forms were reduced by MPA (respectively: −36%, −18% and −28%, $n = 3$ independent experiments, $p < 0.05$ versus control; Fig. 3A). These effects were observable after 3 days of treatment and still fully detectable after 5 days, indicating a sustained inactivation of small GTP-binding proteins. In addition, exposure to MPA caused a significant decrease in total Rac1 protein expression (Fig. 3B; mean variation: $-48 \pm 11\%$, $n = 9$ independent experiments, $p < 0.05$ versus untreated cells). No significant change in RhoA or cdc42 expression level was observed after MPA treatment (data not shown).

3.3. *MPA effects on mesangial cytoskeleton are independent on RhoA but dependent on Rac1*

Our previous results showed that MPA induces an increase in stress fibers (dependent on RhoA activity) and an inhibition in membrane ruffling (dependent on Rac1 activity), all effects that are probably linked to a decrease in intracellular GTP, as suggested by their prevention by guanosine addition. To explore further the role played by RhoA and Rac1 in the alteration of the mesangial phenotype, the consequences of the inhibition of each GTPase on cytoskeleton protein expression were investigated individually.

We have previously shown that MPA increased SMA expression and decreased tubulin expression in IP15 cells [9]. Treatment of cells with the membrane-permeant RhoA inhibitor TAT-C3 (15 μg/mL) had opposite effects on these proteins (Fig. 4): TAT-C3 increased tubulin expression by $45 \pm 24\%$ ($p < 0.05$ versus control), and decreased that of SMA by $-36 \pm 6\%$ ($p < 0.02$ versus control) and that of basic calponin by $-36 \pm 3\%$ ($p < 0.01$ versus control; $n = 8$ from four independent experiments). Moreover, TAT-C3 only partially prevented the effects of MPA. The increase in SMA expression caused by MPA treatment was slightly attenuated in the

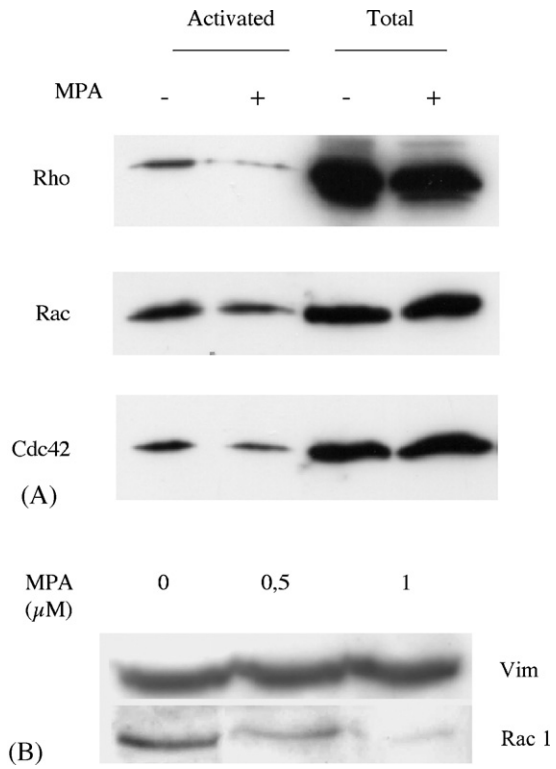

Fig. 3 – MPA prevents activation of Rho, Rac and cdc42. (A) Pull-down assays were performed on IP15 cells treated or not for 5 days with MPA (1 μM). Cells were lysed, and active GTPases were affinity precipitated with GST-RBD-rhotekin or GST-CRIB-PAK, eluted from the beads, and analyzed by Western blotting with the relevant antibodies. For each point, a fraction of the lysate was run to monitor the amount of GTPase before precipitation. (B) IP15 cells were treated for 5 days with or without MPA (0.5 or 1 μM). Western-blot analysis shows expression of Rac1 and that of vimentin (Vim), used as the loading control.

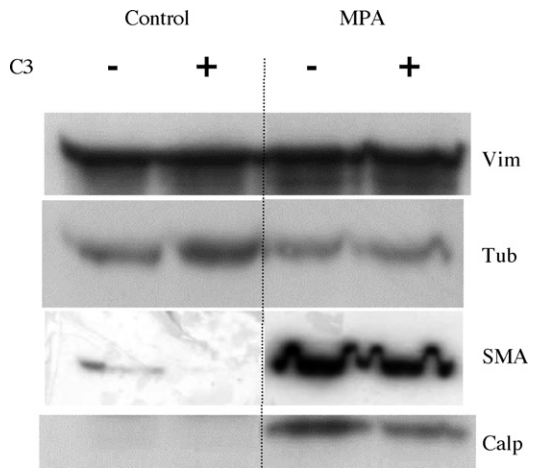

Fig. 4 – Rho inhibition did prevent the effects of MPA on cytoskeletal protein expression. IP15 cells were treated for 3 days with or without MPA (1 μM), and then co-incubated for 18 h with 15 μg/mL of the membrane-permeant RhoA inhibitor TAT-C3 transferase (C3). Western-blot analysis shows expression level of vimentin (Vim, 58 kDa), which is not changed by MPA, and that of tubulin (Tub, 50 kDa), alpha-smooth actin (SMA, 45 kDa), and basic calponin (Calp, 34 kDa).

presence of TAT-C3 (MPA: +866 ± 96%, $p < 0.005$ versus control; MPA + TAT-C3: +657 ± 42%, $p < 0.01$ versus control, $p < 0.05$ versus MPA; $n = 6$ from three independent experiments). The expression of tubulin was decreased by −30 ± 5% by MPA ($p < 0.05$ versus control), an effect that was not significantly altered by co-treatment with TAT-C3 (MPA + C3: −43 ± 14%, $p < 0.05$ versus control, $p = 0.53$ versus MPA alone). Therefore, the decrease in Rho activity is not involved in the changes in cytoskeletal protein expression observed in response to MPA treatment.

On the other hand, overexpression of a wild-type or a constitutively active (QL mutant) form of Rac1 protein decreased basal SMA and calponin expression, while a dominant-negative form of Rac1 (N17 mutant) increased their expression in IP15 cells (Fig. 5A and C). Results from four independent experiments showed that SMA expression (related to vimentin expression) was increased by an average +64% in cells overexpressing the N17 mutant form of Rac1. This effect was found independent on cell mitotic activity, since neither dominant active nor dominant negative mutants of Rac1 had a significant effect on IP15 proliferation (data not shown). At last, down-regulation of Rac1 in IP15 cells using specific siRNA also induced a strong increase in SMA expression (Fig. 5B), confirming the negative effect of Rac on mesangial SMA expression. In four independent experiments, the expression level of Rac1 and SMA were quantitated and compared to that measured in cells transfected with scramble siRNA: the efficiency of Rac1 down-regulation varied from −33.5% to −71% (mean value: −52%), while the increase in SMA expression was comprised between +42% and +63% (mean value: +55%).

MPA treatment of IP15 cells overexpressing wild-type Rac1 resulted in a tubulin down-regulation, whereas SMA and calponin expression levels remained similar to those observed in control cells; only the expression of a constitutively active form of Rac1 (QL mutant), whose activity is less dependent on GTP levels, could prevent the protein changes induced by MPA (Fig. 5C). This result indicates that the inactivation of Rac1 is involved in the mechanisms by which MPA alters mesangial cytoskeletal proteins expresssion.

4. Discussion

The aim of the present study was to explore the mechanisms involved in cytoskeleton alterations induced by long-term treatment of mesangial cells with MPA.

Previous investigations provided evidence that MPA (0.1–10 μmol/L) causes a decrease in intracellular levels of guanine nucleotides not only in leukocytes but also in other cell types [20–22]. A concentration of 0.1 μM guanosine, which corresponds to the physiological plasma level, was found ineffective in preventing the MPA-induced modifications in IP15 cells, whereas a supraphysiological level (100 μM) efficiently antagonised the effects of MPA. This indicates that the inhibition of

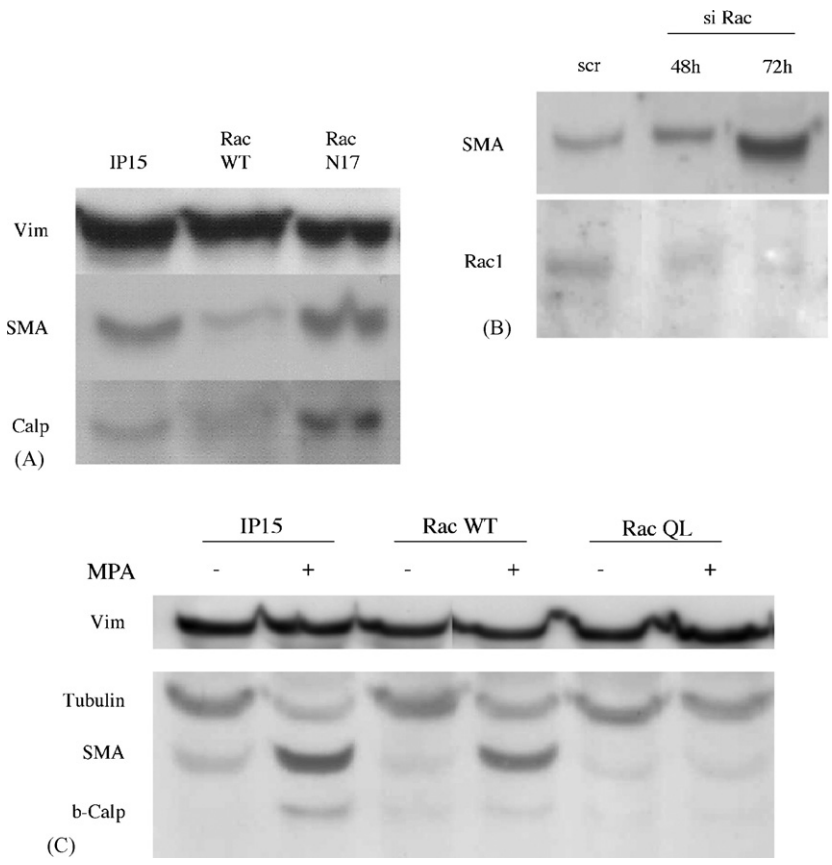

Fig. 5 – Rac regulates SMA and b-calponin expression in IP15 cells. (A) Representative Western-blot analysis showing SMA and basic calponin (calp) expression in non-transformed IP15 cells (IP15), or cells stably transfected with plasmids overexpressing wild-type Rac1 (Rac WT) or an inactive, mutant form of Rac1 (Rac N17). Vimentin (vim) was used as a control of protein loading. (B) Representative Western-blot analysis showing SMA and Rac1 expression in IP15 cells transiently transformed with non-specific, scramble siRNA (scr, 72 h) as a negative control, or specific siRNA against Rac1 (siRAC, 48 and 72 h after transfection). A decrease in Rac1 expression was observable 48 h (−46%) and 72 h (−71%) after transfection, and was accompanied by an increase in SMA expression (48 h: +42%; 72 h: +60%). Equal loading was assessed by Coomassie blue staining of the gel (not shown). (C) Representative Western-blot analysis showing tubulin, SMA and basic calponin (b-calp) expression in non-transformed IP15 cells (IP15), or cells stably expressing wild-type Rac1 (Rac WT) or constitutively active form of Rac1 (QL mutant). Vimentin (vim) was used as a control of protein loading.

IMPDH, which blocks the de novo synthesis of guanine nucleotides, mediated MPA-induced alterations in proliferation, migration, and cytoskeletal protein expression and distribution in human mesangial cells (Figs. 1 and 2). Our results confirm and extend previous findings showing that depletion in guanosine nucleotides is involved in the inhibitory effects of MPA on mesangial proliferation [23] and PDGF-induced MAPK activation [24].

We considered the small GTPases of the Rho family as good candidates to mediate the effects of MPA on mesangial cytoskeleton, since their activation involves guanine nucleotides. In addition, these GTPases regulate the reorganization of the actin cytoskeleton during cell motility, adhesion, and division. In mesenchymal cells, such as mesangial cells, Rac1 and Cdc42 generate lamellipod and pseudopod dynamics at outward edges, favoring migration [25]. Recently, Lakhe-Reddy et al. [26] showed that transduction of human mesangial cells with an inhibitory Rac1 peptide enhanced the organization of SMA-containing fibers. RhoA, on the other hand, stabilizes initial integrin–substrate linkages, increases focal contact size and strength, and further thickens actin filaments through several mechanisms [19,27]. In adhesive cells, active Rho leads to increased adhesiveness, stress fiber formation, and retardation of migration speed.

We have previously shown that in the human mesangial IP15 cell line and *in vitro*, clinically attainable concentrations of MPA induces the formation of thick actin filaments, and alter both membrane ruffling and cell migration [9], all effects that may result from an alteration in Rac1 activity or a stimulation of RhoA activity. In the present study, we showed that MPA decreased the activation of both GTPases (Fig. 3A) and the expression level of Rac1 (Fig. 3B). Thus the effects of MPA on cytoskeletal proteins such as SMA and basic calponin are more likely due to the alteration in Rac1 expression and activity. This finding was confirmed by overexpressing Rac1 in IP15 cells: while the stable expression of either wild-type or constitutively active (QL mutant) Rac1 attenuated SMA expression in non-treated cells, only the expression of the

QL mutant (which activity is not dependent on GTP disponibility) could prevent the induction of SMA or calponin caused by MPA (Fig. 5). Moreover, we can exclude that the decrease in RhoA activity is involved in the mechanism by which MPA stimulates SMA and calponin, since inhibition of Rho by C3-transferase led to a decrease in the expression level of basic calponin in MPA-treated cells (Fig. 4). The absence of a clear effect on SMA expression may be due to the half-life of this protein, which appears to be greater than 24 h [28]. Unfortunately, we could not treat IP15 cells with TAT-C3 for more than 18 h, since extending the treatment period resulted in a dramatic loss of cell viability.

Our results are consistent with recent reports suggesting that small GTPases from the Rho/Rac family can regulate the expression of SMA or SMA-associated proteins in several cell types, including mesangial cells. In LLCPK cells, TGF-beta(1)-induced SMA promoter activation was abrogated by C3 transferase [29]. In rat mesangial cells, the activity of a minimal SMA promoter was potently decreased after treatment with the inhibitor of Rho-kinase Y-27632 [28]. Rho has been described as an activator of the transcription factor SRF that binds to serum-responsive elements present in the promoter of both SMA and basic calponin [12,30,31], suggesting that SRF plays a central role in the mechanisms involved in the regulation of mesangial cytoskeletal protein expression by Rho. However, this hypothesis still remains to be confirmed.

MPA has been shown to reduce expression of Immunoglobulin Fc receptor in various tissues among which the kidney, but mesangial cells have not been specifically studied. However, the general effect of MPA on the cytoskeleton is likely to influence cytoskeleton-dependent mechanisms related to Fc receptors such as Fc receptor-mediated endocytosis, Fc receptor shedding and expression, and therefore to influence Fc receptor-related immunological effects. More generally, the strong MPA-induced inhibition of proliferation of mesangial cells, both *in vitro* and *in vivo* [10,18], should contribute to the reduction of potentially harmful mesangial-dependent immunological effects. These different mechanisms may contribute to the positive effects reported in patients treated with MMF for glomerulonephritides involving mesangial cell proliferation and/or metabolism such as lupus nephritis and IgA nephropathy.

In conclusion, we have demonstrated that mycophenolic acid treatment, followed by a decrease in the intracellular GTP level, alters the expression level and the activity of the Rac1 protein in cultured mesangial cells. The overexpression of SMA and basic calponin induced by MPA can be prevented by overexpressing a constitutively form of Rac1, suggesting that Rac1 down-regulation is involved in the mechanism whereby MPA controls the mesangial cytoskeleton. The hypothesis that MPA may interfer with the small GTPase pathways should be considered while studying cell responses to this drug, not only in mesangial cells but also in other cell types.

REFERENCES

[1] Ducloux D, Fournier V, Bresson-Vautrin C, Rebibou JM, Billerey C, Saint-Hillier Y, et al. Mycophenolate mofetil in renal transplant recipients with cyclosporine-associated nephrotoxicity: a preliminary report. Transplantation 1998;65:1504–6.

[2] Merville P, Berge F, Deminiere C, Morel D, Chong G, Durand D, et al. Lower incidence of chronic allograft nephropathy at 1 year post-transplantation in patients treated with mycophenolate mofetil. Am J Transplant 2004;4:1769–75.

[3] Kramer S, Loof T, Martini S, Ruckert M, Wang Y, Bohler T, et al. Mycophenolate mofetil slows progression in anti-thy1-induced chronic renal fibrosis but is not additive to a high dose of enalapril. Am J Physiol Renal Physiol 2005;289:F359–68.

[4] Rodriguez-Iturbe B, Quiroz Y, Shahkarami A, Li Z, Vaziri ND. Mycophenolate mofetil ameliorates nephropathy in the obese Zucker rat. Kidney Int 2005;68:1041–7.

[5] Romero F, Rodriguez-Iturbe B, Parra G, Gonzalez L, Herrera-Acosta J, Tapia E. Mycophenolate mofetil prevents the progressive renal failure induced by 5/6 renal ablation in rats. Kidney Int 1999;55:945–55.

[6] Badid C, Vincent M, McGregor B, Melin M, Hadj-Aissa A, Veysseyre C, et al. Mycophenolate mofetil reduces myofibroblast infiltration and collagen III deposition in rat remnant kidney. Kidney Int 2000;58:51–61.

[7] Cattran DC, Wang MM, Appel G, Matalon A, Briggs W. Mycophenolate mofetil in the treatment of focal segmental glomerulosclerosis. Clin Nephrol 2004;62:405–11.

[8] Cortes P, Mendez M, Riser BL, Guerin CJ, Rodriguez-Barbero A, Hassett C, et al. F-actin fiber distribution in glomerular cells: structural and functional implications. Kidney Int 2000;58:2452–61.

[9] Dubus I, L'Azou B, Gordien M, Delmas Y, Labouyrie JP, Bonnet J, et al. Cytoskeletal reorganization by mycophenolic acid alters mesangial migration and contractility. Hypertension 2003;42:956–61.

[10] Dubus I, Vendrely B, Christophe I, Labouyrie JP, Delmas Y, Bonnet J, et al. Mycophenolic acid antagonizes the activation of cultured human mesangial cells. Kidney Int 2002;62:857–67.

[11] Nobes CD, Hall A. Rho, Rac and Cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell 1995;81:53–62.

[12] Mack CP, Somlyo AV, Hautmann M, Somlyo AP, Owens GK. Smooth muscle differentiation marker gene expression is regulated by RhoA-mediated actin polymerization. J Biol Chem 2001;276:341–7.

[13] Sauzeau V, Le Mellionnec E, Bertoglio J, Scalbert E, Pacaud P, Loirand G. Human urotensin II-induced contraction and arterial smooth muscle cell proliferation are mediated by RhoA and Rho-kinase. Circ Res 2001;88:1102–4.

[14] Dubus I, Sena S, Bonnet J, Combe C. In vitro prevention of cyclosporin-induced cell contraction by mycophenolic acid. Life Sci 2005;77:3366–74.

[15] Moreau V, Tatin F, Varon C, Genot E. Actin can reorganize into podosomes in aortic endothelial cells, a process controlled by Cdc42 and RhoA. Mol Cell Biol 2003;23:6809–22.

[16] Coso OA, Chiariello M, Yu JC, Teramoto H, Crespo P, Xu N, et al. The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. Cell 1995;81:1137–46.

[17] Tatin F, Varon C, Genot E, Moreau V. A signalling cascade involving PKC Src and Cdc42 regulates podosome assembly in cultured endothelial cells in response to phorbol ester. J Cell Sci 2006;119:769–81.

[18] Allison AC, Eugui EM. Mycophenolate mofetil and its mechanisms of action. Immunopharmacology 2000;47:85–118.

[19] Nobes CD, Hall A. Rho GTPases control polarity, protrusion, and adhesion during cell movement. J Cell Biol 1999;144:1235–44.
[20] Bertalanffy P, Dubsky P, Wolner E, Weigel G. Alterations of endothelial nucleotide levels by mycophenolic acid result in changes of membrane glycosylation and E-selectin expression. Clin Chem Lab Med 1999;37:259–64.
[21] Weigel G, Griesmacher A, Karimi A, Zuckermann AO, Grimm M, Mueller MM. Effect of mycophenolate mofetil therapy on lymphocyte activation in heart transplant recipients. J Heart Lung Transplant 2002;21:1074–9.
[22] Messina E, Gazzaniga P, Micheli V, Barile L, Lupi F, Agliano AM, et al. Low levels of mycophenolic acid induce differentiation of human neuroblastoma cell lines. Int J Cancer 2004;112:352–4.
[23] Hauser IA, Renders L, Radeke HH, Sterzel RB, Goppelt-Struebe M. Mycophenolate mofetil inhibits rat and human mesangial cell proliferation by guanosine depletion. Nephrol Dial Transplant 1999;14:58–63.
[24] Ha H, Kim MS, Park J, Huh JY, Huh KH, Ahn HJ, et al. Mycophenolic acid inhibits mesangial cell activation through p38 MAPK inhibition. Life Sci 2006;79:1561–7.
[25] Keely PJ, Westwick JK, Whitehead IP, Der CJ, Parise LV. Cdc42 and Rac1 induce integrin-mediated cell motility and invasiveness through PI(3)K. Nature 1997;390:632–6.
[26] Lakhe-Reddy S, Khan S, Konieczkowski M, Jarad G, Wu KL, Reichardt LF, et al. Beta8 integrin binds Rho GDP dissociation inhibitor-1 and activates Rac1 to inhibit mesangial cell myofibroblast differentiation. J Biol Chem 2006;281:19688–99.
[27] Clark EA, King WG, Brugge JS, Symons M, Hynes RO. Integrin-mediated signals regulated by members of the rho family of GTPases. J Cell Biol 1998;142:573–86.
[28] Patel K, Harding P, Haney LB, Glass II WF. Regulation of the mesangial cell myofibroblast phenotype by actin polymerisation. J Cell Physiol 2003;195:435–45.
[29] Masszi A, Di Ciano C, Sirokmany G, Arthur WT, Rotstein OD, Wang J, et al. Central role for Rho in TGF-beta1-induced alpha-smooth muscle actin expression during epithelial-mesenchymal transition. Am J Physiol Renal Physiol 2003;284:F911–24.
[30] Liu HW, Halayko AJ, Fernandes DJ, Harmon GS, McCauley JA, Kocieniewski P, et al. The RhoA/Rho kinase pathway regulates nuclear localization of serum response factor. Am J Respir Cell Mol Biol 2003;29:39–47.
[31] Miano JM, Carlson MJ, Spencer JA, Misra RP. Serum response factor-dependent regulation of the smooth muscle calponin gene. J Biol Chem 2000;275:9814–22.