IgA Immune Complex Blunts the Contraction of Cultured Mesangial Cells through the Inhibition of Protein Kinase C and Intracellular Calcium

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Abstract

The effects of IgA immune complex (IgA-IC) on the contractile function of cultured mesangial cells were measured by the changes in planar surface area in response to treatment with agonists. Incubation of mesangial cells with IgA-IC for 24 hours significantly decreased the contractile responses to angiotensin II (10^{-6} M) and phorbol 12-myristate 13-acetate (PMA, 10^{-6} M). Pretreatment of mesangial cells with the protein kinase C (PKC) inhibitor, chelerythrine (10^{-6} M), eliminated the difference in contractile responses to angiotensin II or PMA between the control and IgA-IC groups indicating IgA-IC may inhibit the activity of PKC. The contractile responses to ionomycin were not significantly different between IgA-IC treated and control mesangial cells, suggesting that the contractile machinery is not impaired by IgA-IC. Intracellular calcium, $[Ca^{2+}]_i$ measured by changes in fura-2 level in response to ATP or bradykinin, was significantly inhibited in IgA-IC treated mesangial cells, compared to control cells. In contrast, treatment with thapsigargin did not result in significant differences in $[Ca^{2+}]_i$ between IgA-IC and control mesangial cells, suggesting that a negligible role of endoplasmic reticulum in the effects of IgA-IC. Using PKC specific antibodies, IgA-IC significantly increased the particulate fraction of PKC-ι of mesangial cells to 141±13% of control, without significantly changing the protein content of PKC-α, -δ and -λ in the cytosolic and particulate fractions. In summary, IgA-IC inhibits the contractile responses of cultured mesangial cells to agonists by inhibiting the activation of PKC and $[Ca^{2+}]_i$.

Key Words: mesangial cells, contractility, protein kinase C, intracellular calcium

Introduction

One of the most frequent forms of glomerulonephritis is immunoglobulin A nephropathy (IgAN), which is characterized by prevalent IgA deposits in the mesangial area (11, 15). More than 50% of patients progress toward chronic renal failure. Most patients have a slowly progressive decline in renal function, while a small percentage of them develops renal failure rapidly. In these cases, the frequent

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Received: December 7, 2003; Revised: February 10, 2004; Accepted: February 23, 2004.

clinical findings of proteinuria and hyperfil-tration suggest that hemodynamic mechanisms are involved (11, 15).

In patients with IgAN, serum polymeric IgA is increased, and circulating IgA immuno-complex (IgA-IC) is deposited in mesangial cells with macroscopic or microscopic hematuria (24). The prominent mesangial deposition of IgA-IC leads to the pathogenesis of IgAN with proteinuria and intraglomerular ultrafiltration (11, 15).

Numerous studies have demonstrated that mesangial cells are contractile and resemble vascular smooth muscle cells with respect to their signaling and cytoskeletal responsiveness to hormones (13, 18, 20). Previous studies have demonstrated that the contractile function of mesangial cells regulates glomerular capillary surface area, ultrafiltration coefficient and filtration rate (2, 12, 39). The contraction of mesangial cells involves the interplay of many signal transduction pathways which include both protein kinase C (PKC) and intracellular calcium, $[Ca^{2+}]_i$ (13, 27, 28), and yet the role of PKC and $[Ca^{2+}]_i$ in the effects of IgA-IC is still unclear.

In patients with IgAN, the pathological changes of glomeruli are initially manifested by glomerular hyperfiltration (12, 39). This hyperfiltration of glomerular function could be due to impairment of the contractile function of mesangial cells. To test this hypothesis, we studied the effects of IgA-IC on the contractile function of cultured mesangial cells. Our results demonstrated for the first time that IgA-IC induces hypocontractility of mesangial cells mediated by inhibition of the activation of PKC and $[Ca^{2+}]_i$.

Materials and Methods

Preparation of IgA-IC

IgA-IC were administered by a combination of TEPC-15 hybridoma-derived anti-R36A IgA monoclonal antibody and Pneumococcal C-polysaccharide purified from *Streptococcus pneumoniae*, R36A strain as previously described (30). Preformed IgA-IC were mixed in RPMI-1640 as stock solution by adding IgA (0.5 mg/ml) and R36A (1 mg/ml). Our previous study using this R36A antigen and TEPC-15-IgA in an *in vivo* model demonstrated that this combination induced IgA-IC localization in glomerular mesangial cells (7).

Cell Culture

Mycoplasma-free SV40 CRL-1927 (murine) mesangial cells were obtained from the American Type Culture Collection (Rockville, MD, USA) at passage 27 and were routinely maintained in a 3:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium supplemented with 5% fetal bovine serum and 14 mM HEPES as previously described (31). Cell monolayers were routinely grown to confluence at 37°C in 5% CO_2 before experiments, and all experi-ments were performed between passage 30 and 35 to minimize the effects of phenotypic variation in continuous culture.

Measurements of Mesangial Cell Planar Surface Area

Cultured mesangial cells (1,000 cells/well) were seeded onto plastic 24-well cultures two days before experiments. Twenty-four hours before the contractility experiments, mesangial cells were cultured with or without IgA-IC (containing IgA final concentration 0.005 mg/ml, and R36A 0.01 mg/ml) for 24 hours. A previous study in our laboratory showed that this dosage of IgA-IC is effective in inducing cytokine release in cultured mesangial cells (6).

On the day of the experiment, the 24-well dish was mounted on a heated stage of an inverted light microscope. Using a video camera attached to the microscope, 8-15 cell images per experiment were captured and stored on the hard disk of a microcomputer. Images of the same cell were digitized serially at time intervals from 1 to 5 minutes after treatment. Cell images were calibrated with a ruler (10 μm in length). The perimeter of the individual cell with clearly defined borders was outlined manually and the planar surface area was automatically calculated using WinLab software (DR Instruments, Taipei, Taiwan), which can detect the minimum change of planar surface area to 1 μm^2. The change in mesangial cell planar surface area in response to angiotensin II (10^{-6} M), phorbol 12-myristate 13-acetate (PMA, 10^{-6} M) or calcium ionophore (ionomycin, 10^{-6} M) was observed at 30°C, pH 7.4 with 95% O_2 and 5% CO_2.

To study the involvement of PKC in IgA-IC induced hypocontractility, mesangial cells with or without IgA-IC incubation for 24 hours were contracted with angiotensin II or PMA in the absence or presence of the PKC inhibitor, chelerythrine (10^{-6} M). This concentration of chelerythrine is effective in inhibiting PKC activity in cultured mesangial cells (13). In one of the experiments, IgA-IC were added to determine whether a sudden increase in IgA-IC can induce contraction of mesangial cells.

The change in planar surface area compared to original size was calculated and expressed as a percent of the initial value for each cell. The planar surface area expressed as mean±S.E.M. was determined for each time point.

Pharmacological agents including angiotensin

II, PMA, ATP, bradykinin, thapsigargin and ionomycin were purchased from Sigma Chemical (St. Louis, MO, USA). Chelerythrine (PKC inhibitor) was purchased from Biomol. Res. Lab., Inc. (Plymouth, PA, USA).

Intracellular Calcium Measurements

Intracellular calcium was measured by the ratiometric method in a fura 2-loaded single cell as described previously (8). After two washes with a loading buffer consisting of (mM): 150 NaCl, 5 KCl, 5 glucose, 1 $MgCl_2$, 2.2 $CaCl_2$, and 10 HEPES, pH 7.4, cultured mesangial cells were incubated with 5 μM fura 2-AM in the same buffer at 37°C for 20 min in a coverslip. Then, the fura 2 was removed by washing, and the cells were incubated at 37°C for 10 min to convert the fura 2-ester to the free acid form under the action of nonselective esterase. The coverslip was then mounted in a modified Cunningham chamber (10) attached to the stage of a Olympus IX70 inverted microscope. The fluorescence of the fura 2-loaded cells was monitored using a dual-excitation spectro-fluorometer with a photomultiplier-based detection system (Merlin, Life Science Resources, UK) coupled to the microscope through a fiber-optic cable. Mesangial cells were excited alternatively with 340- and 380-nm light, and the emitted fluorescent light was collected. ATP or bradykinin was administered by adding the pharmacological agent to one side of the Cunningham chamber and draining it through the other side. The fluorescence ratio obtained at 340 and 380 nm (F340/F380) was used as an index of $[Ca^{2+}]_i$. Previous study has demonstrated the F340/F380 ratio correlates linearly with the increase in intracellular calcium (8). Some experiments were performed in calcium-free conditions, with calcium being omitted from the loading buffer and 0.5 mM EGTA added during $[Ca^{2+}]_i$ measurement. All experiments were performed using at least 18 cells. The results were expressed as mean±S.E.M values for the ratio increase.

Cell Fractionation and PKC Immunoblots

PKC isoforms were probed in cytosolic and particulate fractions of growth-arrested mesangial cells after incubation with or without IgA-IC for 24 hours. Cells were washed twice with ice-cold PBS and harvested in 200 μl/100-mm plate buffer A containing (in mM) 1 $NaHCO_3$, 5 $MgCl_2$-$6H_2O$, 50 Tris-HCl, 10 EGTA, 2 EDTA, 1 DTT and 1 phenylmethylsulfonyl fluoride, as well as 25 μg/ml leupeptin and 10 μM benzamidine. The cells were sonicated and passed through a 26-gauge needle three times and incubated on ice for 30 min. Homogenates were centrifuged at 100,000 xg for 1 h at 4°C, and the supernatants were retained as the cytosolic fraction. The pellet was solubilized in 100 μl of 10% SDS, boiled for 10 min, and served as the particulate fraction. Protein concentration was determined using a modified Lowry microassay (Bio-Rad, Hercules, CA, USA). Aliquots were then denatured in 4 X SDS sample buffer, boiled for 5 min, and loaded onto 10% polyacrylamide gel. Samples were electrophoresed at 45 V for 20 min, then at 100 V for 60 min at room temperature. After 3 hours of transfer (225 mA), the polyvinylidene difluoride membrane was blocked overnight at room temperature in PBS buffer containing 10% nonfat milk. The membrane was then exposed to monoclonal anti-PKC-ι (1: 250), -λ (1: 250), -α (1: 1000), or -δ (1: 500) antibodies (BD Biosciences, USA) for 1 h, followed by 1 hour incubation with a horseradish peroxidase-conjugated affinity purified goat anti-mouse IgG antibody (BD Biosciences, USA) diluted at 1: 1000. The blots were rinsed in PBS buffer between each of the preceding steps. The secondary antibody was detected by enhanced chemiluminescence, and the membrane was developed on Kodak Biomax film. Densitometry was performed to analyze the relative density of the protein bands.

3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyl-Tetrazolium Bromide (MTT) Assay

For MTT assay, mesangial cells were cultured in 96-well culture plates at a density of 1000 cells/well with 200 μl of culture medium. After overnight plating, the IgA-IC (IgA: 0.005 mg/ml; R36A: 0.01 mg/ml) were added for 24 to 72 hours. At the time of evaluation of cell growth, 20 μL MTT (5 mg/ml in phosphate-buffered saline solution) was added into each well. After another 3-hour incubation at 37°C, the supernatant was added to 25 μL of Sorenson's glycine buffer and 200 μL of DMSO to dissolve the blue formazan produced by the mitochondrial succinate dehydrogenase of living cultured mesangial cells. Absorbances were measured by a spectrophotometer at a test wavelength of 540 nm (22).

Statistical Analyses

All results were expressed as mean±S.E.M. Statistical analysis was performed using a paired Student's *t*-test. A *P* value less than 0.05 was considered to be statistically significant.

Results

Figure 1 demonstrated the representative contractile responses to the PKC activator, PMA

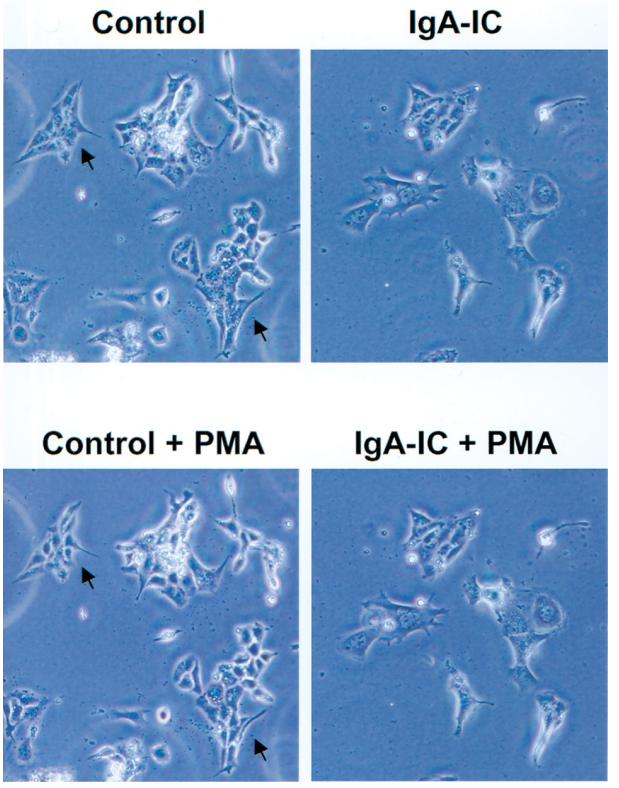

Fig. 1. Representative cell images of cultured mesangial cells (with or without the treatment of IgA-IC for 24 hours) in response to PMA (10^{-6} M) for 30 min. Mesangial cells that had apparent decreasing planar surface area in response to PMA application are indicated by an arrowhead.

(10^{-6} M) in cultured mesangial cells. The planar surface area of control mesangial cells in response to PMA for 30 min was significantly decreased when compared to time "0." However, the planar surface area in response to PMA in IgA-IC (admixture of IgA: 0.005 mg/ml and R36A: 0.01mg/ml) incubated mesangial cells was not significantly altered.

As shown in Figure 2, acute treatment of cultured mesangial cells with IgA-IC induced a contractile response with a significant decrease of the planar surface area to 80±5% of initial at 10 min. However, treatment of cultured mesangial cells with IgA-IC for 24 hours did not significantly change the morphology of cells. The planar surface area of cultured mesangial cells prior to stimulation with agonist was 2511±331 μm^2 for the control group and 2416±406 μm^2 for the IgA-IC treated group (data not shown). There was no significant difference between the surface area of control and IgA-IC treated cells before contractility study.

To determine whether IgA-IC alters the contractile responses of mesangial cells, angiotensin II was acutely added to the medium. Figure 3A shows the change in planar surface area of mesangial cells in

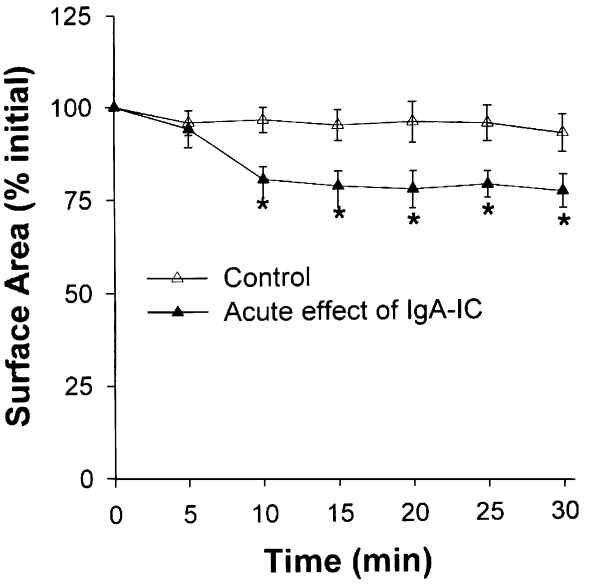

Fig. 2. Change in mesangial cell planar surface area in response to acute treatment with IgA-IC for 30 minutes. Values are mean±S.E.M. Asterisks indicate statistically significant difference between control (open triangles, n = 52) and IgA-IC (closed triangles, n = 48) groups ($P < 0.05$).

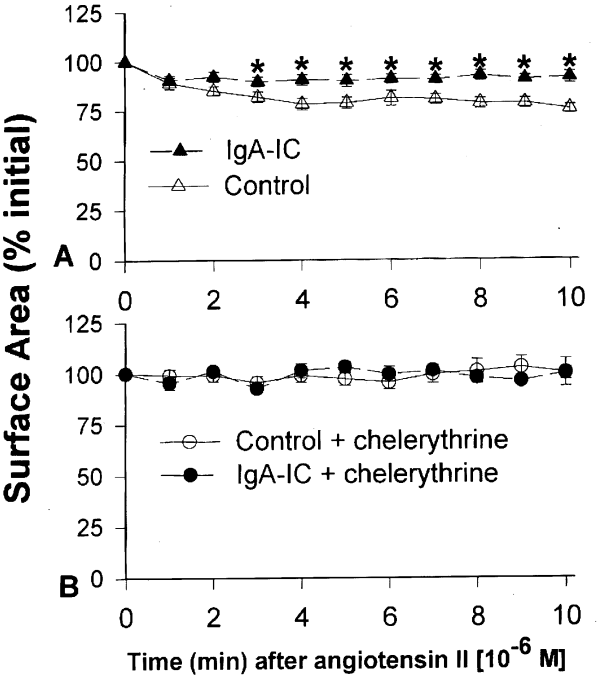

Fig. 3. Change in mesangial cell planar surface area in response to angiotensin II treatment for 10 minutes between control (open triangles and open circles) and IgA-IC (closed triangles and closed circles) incubated (24 hour) cultured mesangial cells with (A) or without (B) the presence of the PKC inhibitor, chelerythrine (10^{-6} M). Values are mean±S.E.M. Asterisks indicate a significant difference between control and IgA-IC groups (n = 45-52, $P < 0.05$).

response to angiotensin II expressed as a percentage of the time 0 value. During stimulation with angiotensin II (10^{-6} M), the surface area decreased over 30 minutes to 74±4% of the initial area. In comparison, the contractile responses to angiotensin II of IgA-IC treated mesangial cells were significantly blunted (planar surface area only decreased to 90±4% of initial). In order to explore the role of PKC in angiotensin II-induced contraction, cultured mesangial cells with and without treatment with IgA-IC for 24 hours were further incubated with 10^{-6} M of chelerythrine (PKC inhibitor) for 20 minutes. As illustrated in Figure 3B, both control and IgA-IC treated mesangial cells did not contract in response to angiotensin II after PKC inhibition (105±8% and 104±5% of initial surface area for control and IgA-IC treated mesangial cells at 30 minutes of stimulation with angiotensin II, respectively).

By applying the PKC activator, PMA, we were able to study if IgA-IC changes PKC-induced contraction in mesangial cells. During PMA (10^{-6} M) application for 30 minutes, the surface area of control mesangial cells decreased to 52±3% of the initial surface area. However, the planar surface area of IgA-IC treated mesangial cells in response to PMA application for 30 minutes was only 75±5 % of the initial surface area (Figure 4A). In a separate experiment, the cultured mesangial cells with or without IgA-IC treatment for 24 hours were further incubated with 10^{-6} M of chelerythrine (a PKC inhibitor) for 20 minutes. Pretreatment of mesangial cells with the PKC inhibitor, chelerythrine, eliminated the difference in contractile responses between control and IgA-IC treated mesangial cells (Figure 4B). These results suggested that PKC does play a role in IgA-IC induced hypocontractility of cultured mesangial cells.

Immunoblot of mesangial cells with PKC-isoforms (-α, -β, -γ, -δ, -ε, -η, -θ, -ι, or -λ) showed that cultured mesangial cells express PKC-α, -δ, -ι, and -λ isoforms at detectable levels. Figure 5 illustrates the particulate and cytosolic expression of PKC-α, -δ, -ι, and -λ isoforms in control and IgA-IC treated mesangial cells. IgA-IC significantly increased particulate PKC-ι content to 141±13 % of control, which was accompanied by a decrease in the cytosolic fraction of PKC-ι. Treatment of mesangial cells with IgA-IC for 24 hours did not significantly change PKC-α, -δ and -λ in the particulate and cytosolic fractions.

In order to test the hypothesis that IgA-IC does not impair the contractile mechanism of mesangial cells, mesangial cells with or without the presence of IgA-IC for 24 hours were stimulated with the calcium ionophore, ionomycin (10^{-6} M). Figure 6 shows that ionomycin caused a near equal decrease in planar surface area of control and IgA-IC treated mesangial

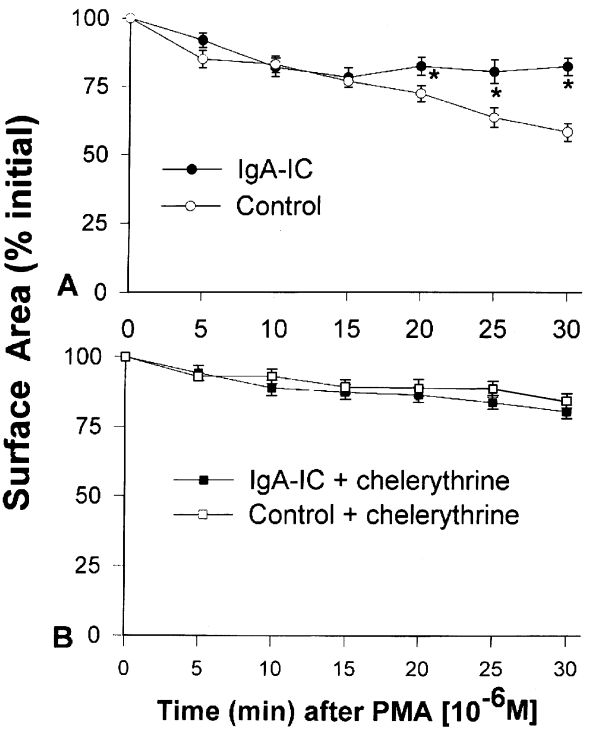

Fig. 4. Change in mesangial cell planar surface area in response to PMA treatment for 30 minutes between control (open circles and open squares) and IgA-IC (closed circles and closed squares) incubated (24 hour) cultured mesangial cells with (B) or without (A) the presence of the PKC inhibitor, chelerythrine (10^{-6} M). Values are mean±S.E.M. Asterisks indicate a significant difference between control and IgA-IC groups (n = 48-54, $P < 0.05$).

cells. The planar surface of control mesangial cells after treatment with ionomycin for 30 minutes was 42±8% of the initial surface area, whereas the planar surface area of IgA-IC treated mesangial cells was 49±7% of the initial surface area. These results suggest that IgA-IC does not influence the contractile machinery of mesangial cells.

To evaluate the extent to which calcium-induced contraction was impaired by IgA-IC treatment, intracellular free calcium, $[Ca^{2+}]_i$, expressed as F340/F380 ratio, was measured in fura 2 loaded mesangial cells. As shown in Table 1, the basal level of F340/F380 ratio in control and IgA-IC treated mesangial cells was 1.6±0.4 and 2.0±0.3, respectively. In response to ATP or bradykinin stimulation in the presence of extracellular calcium, the increase in F340/F380 ratio of IgA-IC treated mesangial cells was significantly less than that of control (Δ1.5±0.5 for ATP and Δ1.4±0.4 for bradykinin in control mesangial cells; and Δ0.5±0.2 for ATP and Δ0.9±0.1 for bradykinin in IgA-IC treated mesangial cells). To study the role of total intracellular pools of calcium in contractility of mesangial cells, ionomycin (10^{-5} M) was applied to the cells without the presence of

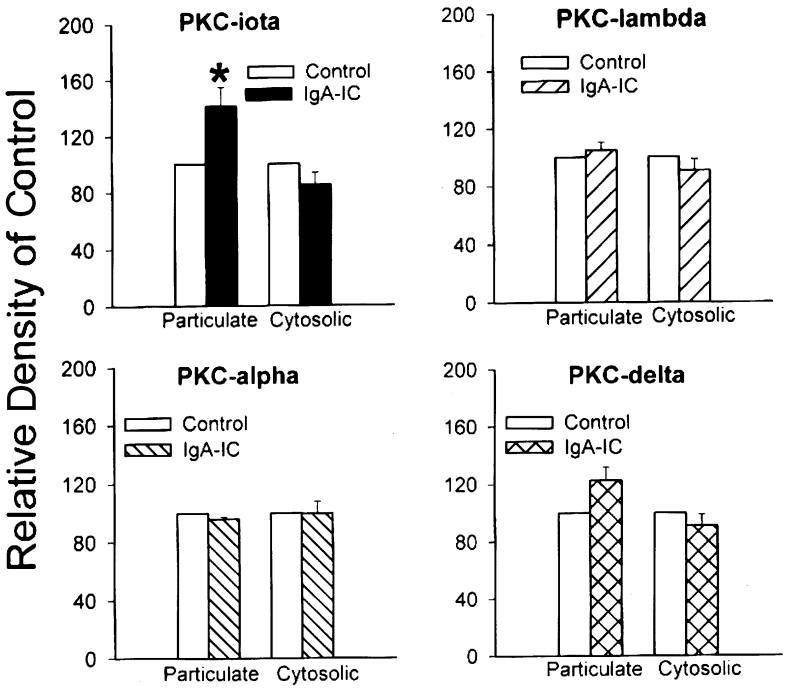

Fig. 5. Effect of IgA-IC on the expression of particulate and cytosolic PKC-ι, -λ, -α, and -δ isoforms in cultured mesangial cells. The histogram represents the mean±S.E.M. of band densitometry data measured in 5 experiments (*$P < 0.05$ vs. control).

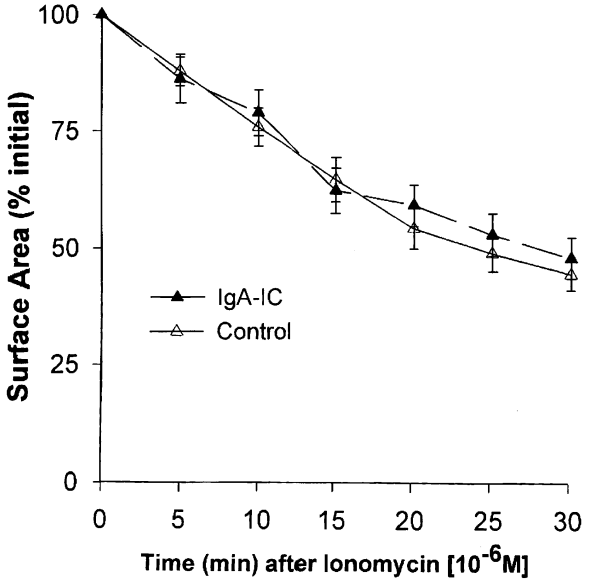

Fig. 6. Change in mesangial cell planar surface area in response to ionomycin (10^{-6} M) treatment for 30 minutes between control (open triangles) and IgA-IC (closed triangles) incubated (24 hour) cultured mesangial cells. Values are mean±S.E.M. Asterisks indicate a significant difference between control and IgA-IC groups (n = 44, $P < 0.05$).

extracellular calcium. The maximum increase in F340/F380 ratio in response to ionomycin was Δ3.8±1.5 for control mesangial cells, and Δ1.2±0.3 for IgA-IC treated mesangial cells, the latter of which was significantly lower than that of the control. Application of thapsigargin (IP_3-independent intracellular calcium releaser) without the presence of extracellular calcium did not significantly alter the maximal increase in F340/F380 ratio between control and IgA-IC treated mesangial cells.

As shown in Figure 7, direct application of IgA-IC to cultured mesangial cells for one to three days did not influence the proliferation of cultured mesangial cells detected by MTT. This result suggests that the dosage of IgA-IC used in this study has no cytotoxic effects on mesangial cells.

Discussion

Our results clearly demonstrated that incubation of cultured mesangial cells with IgA-IC for 24 hours significantly decreases the contractile responses to angiotensin II and PMA. The difference in contractile responses between IgA-IC and control mesangial cells was eliminated by the presence of a PKC inhibitor (chelerythrine), indicating that IgA-IC could inhibit the activity of PKC. IgA-IC also inhibited intracellular calcium mobilization to ATP or bradykinin and may have contributed to the hypo-contractility in IgA-IC treated mesangial cells. These results suggest that IgA-IC could influence the regulatory function of mesangial cells on glomerular capillary surface area mediated by a $[Ca^{2+}]_i$ and PKC dependent pathways.

Table 1. Fura 2, F340/F380 ratio in control and immuno-complex treated mesangial cells.

Treatment	Control	Immuno-complex
Basal	1.6±0.4	2.0±0.3
ATP (10^{-4} M)	Δ1.5±0.5	Δ0.5±0.2*
Bradykinin (10^{-5} M)	Δ1.4±0.4	Δ0.9±0.1*
Ionomycin (10^{-5} M)/Ca^{2+} free	Δ 3.8±1.5	Δ1.2±0.3*
Thapsigargin (10^{-5} M)/ Ca^{2+} free	Δ0.7±0.3	Δ0.5±0.2

Data are mean±S.E.M. of control and immuno-complex treated (24 h) cultured mesangial cells (n = 18-20 cells) in response to different agonists. Cells were bathed in calcium free medium (Ca^{2+} free) prior the application of ionomycin or thapsigargin. Δ indicates the increase in fura 2, F340/F380 ratio from basal level. *P < 0.05 vs. control.

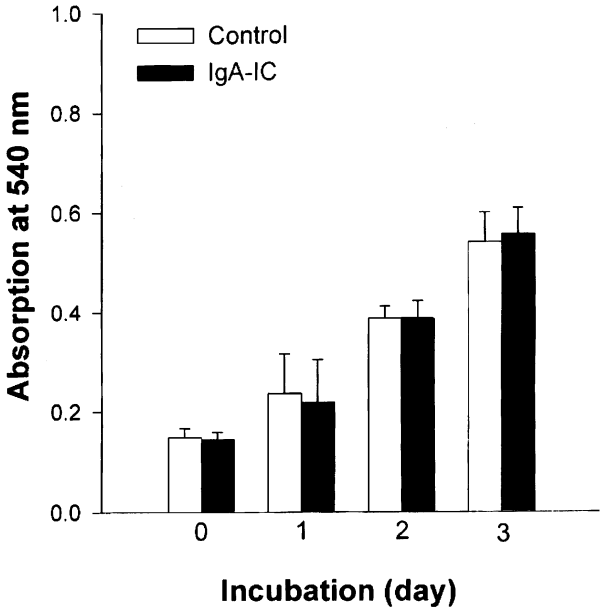

Fig. 7. MTT assay of cultured mesangial cells incubated in control (open bars) or IgA-IC (filled bars) for up to 72 hours. Six different cell cultures were performed as described in Materials and Methods. Values are mean±S.E.M.

IgA nephropathy is initially characterized by IgA-IC deposition in the mesangial area with intraglomerular hypertension and hyperfiltration (24). Morphologically and physiologically, the glomerular mesangial cells are well suited to a capillary surface area-regulating function, as they possess numerous intracellular contractile myofilaments (9, 35). Evidence has now accumulated from a variety of sources suggesting that the glomerular mesangial cell may be the site of the common pathway by which many of the agonists (angiotensin II or ATP) decrease the ultrafiltration coefficient of glomerulus by reducing glomerular capillary surface area and hence single nephron GFR through the contraction of mesangial cells (17, 21, 32). Our measurements of planar surface area were consistent with previous studies that reported a mean decrease of 25-40% in mesangial cell surface area at 30 minutes in response to agonists (13, 18, 27, 29). A decrease in glomerular volume in response to angiotensin II is also seen in isolated glomeruli (18), presumably due to mesangial cell contraction. Thus, IgA-IC deposition in mesangial cells in IgA nephropathy could impair the regulatory function of mesangial cells on glomerular capillary surface area and glomerular filtration coefficient as demonstrated by our contractility study.

Incubation of mesangial cells with IgA-IC for 24 hours induced hypo-contractility to angiotensin II or PMA. This could have been the direct effect of IgA-IC mediation by receptor-couple effects or the effect of cytokine and chemokines release during IgA-IC stimulation (4, 6, 23, 26, 34). Previous studies demonstrated that IgA aggregates and IgA-IC can directly activate mesangial cells via Fc-α receptor (14, 33, 36) and trigger cytokine expression, cell proliferation, and extracellular matrix synthesis (6, 14).

Intracellular free calcium regulates many cellular functions, including contraction. Increase in $[Ca^{2+}]_i$ from intracellular calcium stores (*via* IP_3 generation) and calcium influx *via* receptor-operated calcium channels occur in response to the G protein coupled receptor agonists (ATP or bradykinin) (3, 5, 16, 19). The reduced magnitude of elevation of $[Ca^{2+}]_i$ in response to ATP or bradykinin in IgA-IC treated mesangial cells indicates either the dissociation of receptor-coupled calcium channels or/and the depletion of the intracellular calcium stores is responsible for IgA-IC mediated effects.

We therefore examined the amount of available calcium remaining within the intracellular calcium stores using ionomycin in the absence of extracellular calcium as an index. As shown in Table 1, the increase in F340/F380 ratio induced by ionomycin was signficantly less in the cells that had been subjected to IgA-IC incubation. These results suggest that the decreased response to ATP or bradykinin is at least partially due to the filling state of the intracellular

calcium stores. To further characterize the contribution of endoplasmic reticulum to the $[Ca^{2+}]_i$, we examined the effect of thapsigargin on $[Ca^{2+}]_i$. Thapsigargin blocks calcium loading of the intracellular calcium stores by inhibiting the endoplasmic reticulum calcium pump and depleting the calcium stores of endoplasmic reticulum (5). As shown in Table 1, the thapsigargin-induced change of F340/F380 ratio in control mesangial cells in calcium free buffer was as great as that in IgA-IC incubated mesangial cells. Taken together, these results indicate a negligible contribution of endoplasmic reticulum to the effects of IgA-IC on contractile functions of cultured mesangial cells.

Whether IgA-IC alters the contractile machinery in mesangial cells is unclear. To examine this possibility, the calcium ionophore, ionomycin, was used in a contractile study to equally increase intracellular calcium through extracellular influx of calcium in control and IgA-IC treated mesangial cells independent of PKC activation as previously described (13). In contrast to the responses to angiotensin II or PMA, the contractile responses to ionomycin were equivalent between IgA-IC treated and control mesangial cells, suggesting that IgA-IC does not alter the contractile machinery of mesangial cells.

Intracellular PKC is implicated in the phosphorylation of myosin light chain, which promotes both actin-activated myosin Mg^{2+}-ATPase activity and cross-bridging required for cell motility or contraction (37). The immunoblot data in this study revealed that IgA-IC significantly increases the expression of PKC-ι (atypical PKC) in particulate fraction without significantly changing the expression of PKC-λ (atypical PKC), -α (DAG sensitive/Ca^{2+} dependent PKC), and -δ (DAG sensitive/Ca^{2+} independent) isoforms in particulate and cytosolic fractions of mesangial cells. Since PKC-ι is a DAG insensitive and Ca^{2+} independent isoform, it may not have a significant role in contractile responses of mesangial cells to agonists (1, 25). Further studies are needed to determine if IgA-IC induced increases in the expression of the particulate fraction of PKC-ι have long term effects on the nuclear function of mesangial cells (1, 25).

Although IgA-IC does not alter the PKC sensitive or Ca^{2+} dependent isoforms of PKC at the level of protein content, IgA-IC may still have a significant role in the functional activity of PKC. This role is indicated by our observations that the contractile response of IgA-IC treated mesangial cells to PMA is blunted; and that pretreatment with the PKC inhibitor, chelerythrine, eliminates the effects of IgA-IC on contractile functions of mesangial cells to PMA or angiotensin II. These results suggest that IgA-IC may inhibit the activity of PKC without changing the expression of PKC sensitive or Ca^{2+} dependent isoforms of PKC.

Previous study demonstrated that the existence of different activation pathways for mesangial contraction in that phosphorylation of myosin light chain serine-1, serine-2 and threonine-9 is PKC dependent, whereas the Ca^{2+}/calmodulin-dependent myosin light chain kinase phosphorylates threonine-18 and serine-19 (38). Thus, IgA-IC may inhibit the phosphorylation activity of Ca^{2+}/calmodulin- or/and PKC-dependent myosin light chain kinase.

In conclusion, IgA-IC induced hypocontractility of cultured mesangial cells is likely targeted by the process of activation of intracellular calcium and PKC. Impairment of mesangial contractile capability by IgA-IC may jeopardize glomerular hemodynamics and lead to hyperfiltration and end stage renal disease in IgA nephropathy.

Acknowledgments

The authors thank Mr. Chung-Ding Lee for his excellent technical assistance. This study was supported by grants from National Science Counsel, NSC 92-2314-B-016-013, and from National Health Research Institute, NHRI-EX91-9137SN, Taiwan, R.O.C.

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