IGF-I and insulin amplify IL-1β-induced nitric oxide and prostaglandin biosynthesis

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Guan, Zhonghong, Shaavhree Y. Buckman, Lisa D. Baier, and Aubrey R. Morrison. IGF-I and insulin amplify IL-1β-induced nitric oxide and prostaglandin biosynthesis. Am. J. Physiol. 274 (Renal Physiol. 43): F673–F679, 1998.—The inflammatory cytokine interleukin-1β (IL-1β) induces both cyclooxygenase-2 (Cox-2) and the inducible nitric oxide synthase (iNOS) with concomitant release of PGs and nitric oxide (NO) by glomerular mesangial cells. In our current studies, we determine whether insulin and IGF-I are involved in the signal transduction mechanisms resulting in IL-1β-induced NO and PGE2 biosynthesis in renal mesangial cells. We demonstrate that both insulin and IGF-I increase IL-1β-induced Cox-2 and iNOS protein expression, which in turn enhance PGE2 and NO production. Our data also indicate that both insulin and IGF-I enhance IL-1β-induced p38 mitogen-activated protein kinase (MAPK) phosphorylation and SAPK activation. These findings implicate the possible role of the MAPK pathway in mediating the effects of insulin and IGF-I on the upregulation of cytokine-stimulated NO and PG biosynthesis. Together, our results indicate that IGF-I and insulin may function to modulate the renal inflammatory process.

p38 mitogen-activated protein kinase; stress-activated protein kinase; mesangial cells; cyclooxygenase; nitric oxide synthase

INTERLEUKIN-1 (IL-1) is a potent immunoregulatory and proinflammatory cytokine secreted by a variety of cells in response to infection, activated lymphocyte products, microbial toxins, inflammation, and other stimuli (9, 10). In glomerular inflammation, infiltrating macrophages produce IL-1, which activates renal mesangial cells and promotes glomerular damage. IL-1 signaling in mesangial cells stimulates both PG and nitric oxide (NO) pathways and increases PG and NO production by inducing both cyclooxygenase-2 (Cox-2) and the inducible NO synthase (iNOS) (36, 37). NO is recognized as an important effector molecule that mediates cell-cell communication in many mammalian systems. NO is derived from the amino acid L-arginine in an unusual oxidative reaction that consumes molecular oxygen and reducing equivalents in the form of NADPH. The inducible NOS (iNOS) has been identified in several cell types, including macrophages, vascular smooth muscle cells, and renal mesangial cells (19, 23–25, 29, 30, 32, 40). It is highly regulated by cytokines, some of which promote or inhibit the induction of this enzyme. Stimulatory cytokines such as IL-1 and tumor necrosis factor-α increase iNOS mRNA by transcriptional activation. Once iNOS is induced, it remains activated for hours or days and produces large amounts of NO which contributes to cell and tissue regulation and damage. However, iNOS gene expression, mRNA stability, protein synthesis, and degradation are all amenable to modification by cytokines or other agents such as growth factors (30).

The cyclooxygenases are another important group of enzymes involved in many inflammatory processes. Isoforms Cox-1 and Cox-2 are key enzymes that convert arachidonic acid to PGs. Cox-1 is constitutively expressed in most tissues such as kidney, stomach, vascular smooth muscle, and platelets, where PGs are thought to play "housekeeping" functions, such as cytoprotection of the gastric mucosa, regulation of renal blood flow, platelet aggregation, and maintenance of normal physiological processes (22). In contrast, Cox-2 is normally undetectable in most tissues, but can be rapidly induced in certain cell types by various proinflammatory or mitogenic agents. This inducible enzyme is thought to be involved in inflammation, cellular differentiation, and mitogenesis via the release of proinflammatory PGs. Surprisingly, mice lacking Cox-2 have normal inflammatory responses but develop severe nephropathy that causes progressive renal failure as the animal ages, suggesting that Cox-2 may be critical for maintaining normal kidney development, differentiation, and function (28). We have previously described that IL-1β induces Cox-2 but not Cox-1 followed by increases in PG production in renal mesangial cells. The secretion of PG, in turn, regulates mesangial cell and macrophage function (36, 37).

IL-1 stimulation activates a family of protein kinases known as the mitogen-activated protein kinases (MAPKs). At least four genetically distinct MAPK pathways, which are functionally independent and regulated by distinct protein cascades, have been identified in mammalian cells, including the extracellular signal-regulated kinase (ERK1 and ERK2), ERK5, stress-activated protein kinase (SAPK), also called c-jun amino-terminal kinases (JNK), and p38 MAPK. These kinases are activated by distinct upstream dual specificity kinases [MAPK kinase (MKK)/MEK], which phosphorylate both threonine and tyrosine in the regulatory Thr-X-Tyr motif present in all MAPKs. Once activated, these MAPKs then phosphorylate and activate their specific substrates on serine and/or threonine residues and produce their effects on downstream targets (3, 4, 39). Previous studies have demonstrated that IL-1β activates both SAPK and p38 MAPK in renal mesangial cells. These two protein kinase signaling cascades may be involved in the regulation of NO and PG biosynthesis triggered by IL-1β (14, 15).

Insulin and insulin-like growth factor I (IGF-I) are two structurally related homologous polypeptide hor-
mones that produce pleiotropic effects in target tissues, including effects on metabolism and growth. Receptors for insulin and IGF-I also display a high degree of structural homology. Both receptors contain a tyrosine-specific protein kinase domain that plays a pivotal role in the intracellular signaling. As a first step in initiating responses, insulin and IGF-I bind to their specific plasma membrane receptors. Immediately after binding, the receptors for insulin and IGF-I undergo auto-phosphorylation on tyrosine residues. Autophosphorylation increases the tyrosine kinase activity of the receptor, which in turn phosphorylates several cellular substrates, leading to cascades of secondary phosphorylation and dephosphorylation (7, 8, 13, 33, 34). Although most of the growth-promoting effects appear to be mediated by the IGF-I receptor and the metabolic effects by the insulin receptor, the biological responses to insulin and IGF-I at the cellular level largely overlap. This may be due to the low-affinity binding of ligands to alternative receptors and the use of similar intracellular signaling pathways. Thus stimulation of IGF-I receptors may be observed in vitro with high nonphysiological levels of insulin. This may occur in clinical conditions characterized by severe hyperinsulinemia as a pathogenic mechanism of altered tissue proliferation in non-insulin targets such as the arterial wall (12, 18, 38). The glomerular mesangial cell is one of the important sites of renal synthesis and secretion of IGF-I. Since mesangial cells do not appear to have high-affinity insulin receptors, it is believed that both insulin and IGF-I bind to the IGF-I receptor on mesangial cells, which in turn induces mesangial proliferation and extracellular matrix synthesis (1, 2, 5, 6, 11, 21). Because of the important role of mesangial proliferation and extracellular matrix synthesis in glomerular inflammation, it is intriguing to determine how insulin and IGF-I may function in the renal inflammatory response.

In our present studies, we investigate whether insulin and IGF-I exert their effects on the signal transduction mechanisms of IL-1β-induced NO and PGE2 biosynthesis in mesangial cells. We find that both insulin and IGF-I increase IL-1β-induced PGE2 and NO biosynthesis in glomerular mesangial cells. In addition, we demonstrate that insulin and IGF-I enhance IL-1β-induced SAPK and p38 activity in this cell type. This data suggests that insulin and IGF-I are involved in regulating the renal inflammatory process by regulating NO and PGE2 production stimulated by IL-1β, which may be mediated by MAPK signal transduction mechanisms.

**METHODS**

Materials. IL-1β (100 half-maximal units/ng) was purchased from Boehringer Mannheim (Indianapolis, IN). Insulin and IGF-I were from Eli Lilly (Indianapolis, IN) and Genentech (San Francisco, CA), respectively. PGE2 was from Sigma (St. Louis, MO). Fetal bovine serum was purchased from Life Technologies (Gaithersburg, MD). Polyclonal rabbit IgG antibodies against iNOS, Cox-2, and phospho-specific p38 were from Transduction Laboratories (Lexington, KY), Cayman Chemicals (Ann Arbor, MI), and New England BioLabs (Beverly, MA), respectively. pET28cΔ, a histidine-tagged fusion protein expression plasmid that encodes amino acids 1–79 of N-H2 terminal c-Jun, was generously provided by Dr. Maryann Gruda (Department of Molecular Biology, Bristol Myers Squibb Pharmaceutical Research Institute, Princeton, NJ). His-c-jun-(1–79) was expressed as a histidine-tagged fusion protein in Escherichia coli NovablaDE (DE3) and purified by His-bind resin (Novagen) (25).

Cell culture. Primary mesangial cell cultures were prepared from male Sprague-Dawley rats as previously described (2). Cells were grown in RPMI-1640 medium supplemented with 15% heat-inactivated FCS, 0.3 IU/ml insulin, 100 U/ml penicillin, 100 µg/ml streptomycin, 250 µg/ml amphotericin B, and 15 mM HEPES, pH 7.4. All experiments were performed with confluent cells grown in 25-cm² or 75-cm² flasks and used at passages 3–8. Serum was reduced from 15% to 5%, and insulin was removed 24 h before experiments.

Western blot analysis. Confluent cells incubated in RPMI-1640 media containing 5% FCS were treated with IL-1β, with or without other pharmacological compounds. Cells were washed with ice-cold phosphate buffer and lysed in 0.5 ml of whole cell extract buffer (WCE) [HEPES-NaOH (pH 7.7), 0.3 M NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.1% Triton X-100, 0.5 mM dithiothreitol (DTT), 20 mM β-glycerophosphate, 100 µM NaVO4, 2 µg/ml leupeptin, and 100 µg/ml PMSF] in which 6× Laemmli sample buffer was added before heating. After boiling for 5 min, equal amounts of protein were run on 7.5–12% SDS-PAGE. Proteins were transferred to polyvinylidene difluoride membranes (Immobilon-P; Millipore, Bedford, MA). The membranes were saturated with 5% fat-free dry milk in Tris-buffered saline (50 mM Tris, pH 8.0, 150 mM NaCl) with 0.05% Tween-20 (TBS-T) 1 h at room temperature. Blots were then incubated overnight with anti-iNOS, Cox-2, or phospho-specific p38 MAPK antibodies at 1:1,000 dilution in 5% albumin TBS-T solution. After five washes with 5% milk TBS-T solution, blots were further incubated for...
1 h at room temperature with the goat anti-rabbit IgG antibody coupled to horseradish peroxidase (Amersham, Arlington Heights, IL) at 1:2,000 dilution in above solution. Blots were washed five times again in TBS-T before visualization. An enhanced chemiluminescence kit (ECL, Amersham) was used for detection.

In-gel protein kinase assays. After experimental maneuvers, various harvested cells were solubilized in WCE buffer. Protein kinase assays were performed using modifications of our previous procedures. Briefly, SDS-polyacrylamide was polymerized in the presence or absence of 200 µg/ml of His-c-jun (1–79). After electrophoresis, SDS was removed by incubation in 20% isopropanol in 50 mM Tris·HCl (pH 8.0) for 1 h. The gel was then washed for 1 h with 1 mM DTT and 50 mM Tris·HCl (pH 8.0). To denature the proteins, gels were incubated in 6 M guanidine-HCl, 20 mM DTT, 2 mM EDTA, and 50 mM Tris·HCl (pH 8.0) for 1 h. Proteins were then renatured by incubation overnight in 1 mM DTT, 0.04% Tween-20, and 50 mM Tris·HCl (pH 8.0). For the protein kinase assays, gels were equilibrated for 1 h in kinase buffer containing 1 mM DTT, 0.1 mM EGTA, 20 mM MgCl₂, 40 mM HEPES-NaOH (pH 8.0), and 100 µM NaVO₄. The kinase reaction was carried out for 1 h in kinase buffer with 30 µM ATP and 5 µCi/ml of [γ-³²P]ATP. Finally, the gels were washed extensively in 5% trichloroacetic acid and 1% sodium pyrophosphate until washes were free of radioactivity. Autoradiography of dried gels was performed at –80°C.

PGE₂ determination. PGE₂ in the culture media was determined by stable isotope gas chromatography-mass spectrometry (GC-MS) as described previously (14). At the end of predetermined times, medium was removed and spiked with 25 ng tetradeterated PGE₂ (d₄-PGE₂). The media was then acidified to pH 3.5, and PGE₂ was extracted with 1-ml octadeyl columns (Baker, Sanford, ME). Extracts were derivatized for GC-MS analysis. The samples were analyzed as the pentafluorobenzyl ester trimethylsilyl ether by negative ion chemical ionization using methane as the reagent gas. Ions monitored were m/z 524 (d₀-PGE₂) and m/z 528 (d₄-PGE₂). Mass spectrometry was performed on a Hewlett-Packard 5985 spectrometer using a 25-m Ultra 1 capillary column (Hewlett-Packard, Palo Alto, CA), and data collection and analysis were performed using Vector 2 software (Teknivent, St. Louis, MO). PGE₂ production was normalized for protein as determined by the micro bicinchoninic acid assay.

Nitrite determination. The conditioned incubation medium was collected, and nitrite content was measured by the addition of Griess reagent (1% sulfanilamide-0.1% naphthylethylenediamine dihydrochloride in 2% phosphoric acid). The absorbance at 550 nm was measured, and the amount of nitrite was obtained by extrapolation from a standard curve using sodium nitrite as a standard. The nitrite production was corrected for protein determined as described above.

Statistical analysis. Data were expressed as the means ± SE. Statistical analysis was performed by using a paired or Fig. 2. Effects of IGF-I and insulin (Ins) on IL-1β-induced PGE₂ production. A: mesangial cells were treated with or without 100 U/ml of IL-1β in presence of different concentration of insulin for 24 h. PGE₂ levels in culture media were determined. Results are means ± SE (n = 3). B: mesangial cells were treated with or without 100 U/ml of IL-1β in presence of different concentrations of IGF-I for 24 h. PGE₂ levels in culture media were determined. Results are means ± SE (n = 3).

Fig. 3. Effects of IGF-I and insulin on IL-1β-induced cyclooxygenase-2 (Cox-2) expression. Mesangial cells were treated with or without 100 U/ml of IL-1β in presence of IGF-I (100 nM) and insulin (0.3 IU/ml) for 0–36 h. Western blot assay was performed with anti-Cox-2 as the primary antibody. Positions of Cox-2 are indicated.
A difference with a \( P \) value of 0.05 was considered statistically significant.

### RESULTS

#### Effects of insulin and IGF-I on \( \text{PGE}_2 \) biosynthesis.

To determine whether insulin and IGF-I regulate IL-1\( \beta \)-stimulated \( \text{PGE}_2 \) production, we studied the effects of these two proteins on IL-1\( \beta \)-induced \( \text{PGE}_2 \) biosynthesis in renal mesangial cells. Time course experiments demonstrated that both insulin (0.3 IU/ml) and IGF-I (100 nM) increased IL-1\( \beta \)-induced \( \text{PGE}_2 \) production by mesangial cells incubated in the presence of 5% serum (Fig. 1). Both insulin (0.003–30 IU/ml) and IGF-I (0.01–1,000 nM) dose-dependently enhanced IL-1\( \beta \)-induced \( \text{PGE}_2 \) production (Fig. 2, A and B). Western blot analysis further illustrated that both insulin and IGF-I significantly increased IL-1\( \beta \)-stimulated Cox-2 protein expression. The combination of insulin with IGF-I resulted in increased Cox-2 expression when compared with either agent used alone (Fig. 3). However, these peptides by themselves failed to significantly affect \( \text{PGE}_2 \) production (Fig. 1 and 2) and Cox-2 protein expression (data not shown). The above observations suggest that both insulin and IGF-I enhance the signaling pathway invoked by IL-1\( \beta \), which results in the increase of Cox-2 and \( \text{PGE}_2 \) production.

#### Effects of insulin and IGF-I on NO production.

To investigate whether insulin and IGF-I regulate NO synthesis induced by IL-1\( \beta \), we tested the effects of these two drugs on IL-1\( \beta \)-induced NO biosynthesis. Both insulin and IGF-I dose-dependently increased IL-1\( \beta \)-induced nitrite production (Fig. 4, A and B). Time course data also demonstrated that both insulin and IGF-I (0.3 IU/ml and 100 nM, respectively) increased IL-1\( \beta \)-induced nitrite production secreted by mesangial cells (Fig. 5). Furthermore, Western blot analysis demonstrated that both insulin and IGF-I at the above concentrations significantly increased iNOS protein expression stimulated by IL-1\( \beta \). The combination of
insulin with IGF-I resulted in increased iNOS expression when compared with either agent used alone (Fig. 6). By themselves, insulin and IGF-I did not induce significant nitrite production (Fig. 4 and 5) and iNOS expression (data not shown). These results suggest that both insulin and IGF-I upregulate IL-1β-induced NO biosynthesis in mesangial cells.

Effects of insulin and IGF-I on p38 MAPK phosphorylation. To demonstrate whether insulin and IGF-I modulate IL-1β-induced p38 MAPK, we tested the effects of insulin and IGF-I on p38 MAPK tyrosine phosphorylation. Our previous data have demonstrated that IL-1β-induced phosphorylation of p38 MAPK correlates with p38 MAPK activation (14). In the current experiments, we assessed p38 MAPK phosphorylation by Western blot assay using an anti-phospho-specific p38 MAPK antibody to reflect the p38 MAPK activation. Our experiments demonstrated that both insulin (0.3 IU/ml) and IGF-I (100 nM) by themselves had minimal effects on p38 MAPK phosphorylation. As previously observed, IL-1β (100 U/ml), significantly increased p38 MAPK phosphorylation in insulin-starved cells. Furthermore, both insulin and IGF-I markedly upregulated IL-1β-induced P38 MAPK phosphorylation (Fig. 7, A and B). These data indicate that p38 MAPK may function to modulate the ability of insulin and IGF-I to enhance IL-1β-induced NO and PG synthesis.

Fig. 6. Effects of IGF-I and insulin on IL-1β-stimulated NO and PG synthesis. A: mesangial cells were treated with or without 100 U/ml of IGF-I in presence of IL-1β for 0–60 min. Western blot assay was performed with anti-phospho-specific p38 MAPK as the primary antibody. Positions of phosphorylated p38 MAPK (pp38) are indicated. B: mesangial cells were treated with or without 100 U/ml of insulin in presence of IL-1β for 0–60 min. Western blot assay was performed with anti-phospho-specific p38 MAPK as the primary antibody. Positions of phosphorylated p38 MAPK (pp38) are indicated.

Fig. 7. Effects of IGF-I and insulin on IL-1β-stimulated p38 MAPK phosphorylation. A: mesangial cells were treated with or without 100 U/ml of IGF-I in presence of IL-1β for 0–60 min. Western blot assay was performed with anti-phospho-specific p38 MAPK as the primary antibody. Positions of phosphorylated p38 MAPK (pp38) are indicated. B: mesangial cells were treated with or without 100 U/ml of insulin in presence of IL-1β for 0–60 min. Western blot assay was performed with anti-phospho-specific p38 MAPK as the primary antibody. Positions of phosphorylated p38 MAPK (pp38) are indicated.

Effects of insulin and IGF-I on SAPK kinases activity. To investigate whether insulin and IGF-I regulate IL-1β-induced SAPK activation, we studied the effects of insulin and IGF-I on SAPK activity (data not shown). However, as shown in Fig 8, IL-1β significantly increased SAPK activity in insulin-starved cells. Furthermore, both insulin and IGF-I significantly increased IL-1β-induced p45 and p54 SAPK activity. These data indicate that insulin and IGF-I may upregulate IL-1β-induced SAPK activity via activation of the SAPK pathway.

DISCUSSION
The inflammatory cytokine IL-1 is involved in several pathological processes of renal glomeruli. IL-1 induces a variety of biochemical and functional responses in mesangial cells. The IL-1-activated phenotype is believed to play an important role in the progression of glomerular inflammatory injury. Our laboratory previously reported that in primary cultures of mesangial cells, IL-1β induces iNOS and Cox-2 expression with concomitant release of NO and prostaglandins. The activation of these key mediators may provide an important mechanism mediating renal inflammation (35–37).

Previous studies have demonstrated that glomerular mesangial cells are the important site for synthesis, secretion, and binding IGF-I in the kidney (2, 5, 6). Receptor binding assays have shown that renal mesangial cells have a high-affinity IGF-I receptor and a low-affinity insulin receptor. This may suggest that the effects of insulin and IGF-I are mediated by the higher-affinity IGF-I receptors (1). Renal IGF-I levels are increased in some experimental models of chronic renal failure, and IGF-I has been implicated in enhancing extracellular matrix synthesis by renal cells (21, 26, 27). IGF-I has also been shown to increase procollagen levels and increases collagen synthesis in association with mesangial cell hyperplasia and glomerulosclerosis. These findings have been described in models of mesangial-proliferative glomerulonephritis and experimental diabetic nephropathy, as well as focal and segmental glomerulosclerosis (11). More interestingly, recent data demonstrated that IGF-I improves renal}

#### Fig. 6. Effects of IGF-I and insulin on IL-1β-induced inducible nitric oxide synthase (iNOS) expression. Mesangial cells were treated with or without 100 U/ml of IL-1β in presence of IGF-I (100 nM) and insulin (0.3 IU/ml) for 0–36 h. Western blot assay was performed with anti-iNOS as the primary antibody. Positions of iNOS are indicated.
function in cases of acute or chronic renal failure. The mechanism of this action likely involves the enhancement of renal blood flow and glomerular filtration rate (16, 20, 31). The hemodynamic action of IGF-I is blocked by indomethacin administration, suggesting the modulatory role of vasodilatory eicosanoids (17). In our current study, we demonstrate that IGF-I and insulin increase IL-1β-induced iNOS and Cox-2 expression, which in turn enhances NO and PGE2 production. Specifically, our findings suggest that IGF-I and insulin may amplify the production of inflammatory mediators such as NO and PGE2.

Intracellular signaling mechanisms by which IL-1β induces iNOS and Cox-2 expression are incompletely understood. The MAPK family of serine/threonine protein kinases is a vital signaling mechanism that transmits signals from the cell surface to the nucleus to ultimately regulate gene expression. At least three subgroups of MAPKs have been identified, including ERKs, SAPKs, and p38 MAPK. We have previously demonstrated that IL-1β stimulation of renal mesangial cells mediates PGE2 and NO production, as well as Cox-2 and iNOS expression with concomitant activation of p38 MAPK- and SAPK-mediated signaling mechanisms (14, 15). Furthermore, by using a p38 MAPK inhibitor, we demonstrated that the p38 MAPK pathway is an important mechanism to mediate Cox-2 and iNOS expression as well as PGE2 and NO production induced by IL-1β. These data suggest the role of p38 MAPK in the modulation of PG and NO biosynthesis in response to the amplification of renal inflammatory stimuli (14, 15). Given these results, we further questioned whether the influence of IGF-I and insulin on IL-1β-induced NO and PG production was also mediated by the MAPK pathway. Our experiments demonstrated that both IGF-I and insulin can significantly increase IL-1β-induced SAPK and p38 MAPK activity, suggesting that SAPK/JNK and p38 MAPK signaling pathways may mediate the effects of IGF-I and insulin on IL-1β-induced Cox-2 and iNOS expression. Interestingly, there is a contradiction between this current study and the previous study in which we have reported that p38 MAPK downregulates NO while upregulating PGE2 synthesis (14). However, one of the important differences between these two experiments is the JNK pathway. By using a p38 inhibitor, the earlier study showed that p38 downregulates NO while upregulating PGE2 synthesis. However, in this study, insulin and IGF-I upregulate both p38 and JNK pathways. Thus far, no published data have demonstrated the effects of the JNK pathway on IL-1-induced PGE2 and NO biosynthesis. Interestingly, some of our recent unpublished studies using dominant negative JNK illustrate that JNK upregulates both PGE2 and NO synthesis. So our current hypothesis suggests that upregulation of JNK by insulin or IGF-I may be an important signaling mechanism contributing to amplification of NO induced by IL-1. Furthermore, insulin and IGF-I can activate ERK1 and ERK2, which creates another layer of complexity.

In summary, our current study illustrates that both IGF-I and insulin increase IL-1β-induced Cox-2 and iNOS protein expression, which in turn, increases PGE2 and NO biosynthesis in glomerular mesangial cells. These results suggest that insulin and IGF-I can influence the inflammatory process by upregulating cytokine-stimulated NO and PGE2 synthesis. In addition, this study further demonstrates that insulin and IGF-I augment IL-1β-induced SAPK and p38 MAPK activity in this cell type. These results implicate activation of MAPK pathway in mediating the effects of IGF-I and insulin. Clearly, further study is necessary to determine the intercellular signaling mechanism by which insulin and IGF-I can potentiate the action of IL-1. Together, our results suggest a role for IGF-I and insulin in modulating the renal inflammatory process.

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REFERENCES


