Endotoxin and cisplatin synergistically stimulate TNF-α production by renal epithelial cells

Ganesan Ramesh, Scott R. Kimball, Leonard S. Jefferson, and W. Brian Reeves

Division of Nephrology and Department of Cellular and Molecular Physiology, Pennsylvania State University College of Medicine, Hershey, and Lebanon Veterans Affairs Medical Center, Lebanon, Pennsylvania

Submitted 19 July 2006; accepted in final form 5 October 2006

Ramesh G, Kimball SR, Jefferson LS, Reeves WB. Endotoxin and cisplatin synergistically stimulate TNF-α production by renal epithelial cells. Am J Physiol Renal Physiol 292: F812–F819, 2007. First published October 10, 2006; doi:10.1152/ajprenal.00277.2006.—Acute renal failure often occurs in the clinical setting of multiple renal insults. Tumor necrosis factor-α (TNF-α) has been implicated in the pathogenesis of cisplatin nephrotoxicity, ischemia-reperfusion injury, and endotoxin-induced acute renal failure. The current studies examined the interactions between cisplatin and endotoxin with particular emphasis on TNF-α production. Treatment of cultured murine proximal tubule cells (TKPTS cells) with cisplatin resulted in a modest production of TNF-α, while treatment with endotoxin did not result in any TNF-α production. However, the combination of cisplatin and endotoxin resulted in large amounts of TNF-α synthesis and secretion. The stimulation of TNF-α production was dependent on cisplatin-induced activation of p38 MAPK and was associated with phosphorylation of the translation initiation factor eIF4E and its upstream kinase Mnk1. Inhibition of p38 MAPK and, to a lesser extent, ERK, reduced cisplatin+endotoxin-stimulated TNF-α production and phosphorylation of Mnk1 and eIF4E. Synergy between cisplatin and endotoxin was also observed in certain tumor cell lines, but not in macrophages. In macrophages, in contrast to TKPTS cells, endotoxin alone activated p38 MAPK and stimulated TNF-α production with no added impact by cisplatin. The combination of cisplatin and endotoxin did not result in synergistic production of other cytokines, e.g., MCP-1 and MIP2, by TKPTS cells. In summary, these studies indicate that cisplatin sensitizes renal epithelial cells to endotoxin and dramatically increases the translation of TNF-α mRNA in a p38 MAPK-dependent manner. These interactions between cisplatin and endotoxin may be relevant to the pathogenesis of cisplatin nephrotoxicity in humans.

Address for reprint requests and other correspondence: W. B. Reeves, Div. of Nephrology, Rm. C5830, Pennsylvania State College of Medicine, 500 University Dr., Hershey, PA 17033 (e-mail: wreeves@psu.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
METHODS

Cell culture. Murine proximal tubule cells (TKPTS cells; kindly provided by Dr. E. Bello-Reuss, University of Texas Medical Branch, Galveston, TX) were cultured in DMEM/F12 supplemented with glutamine, 7.5% FBS, and antibiotics. Cells were grown to confluence at 37°C in 5% CO2. All experiments were carried out in serum-containing medium. Cells were treated with different concentrations of cisplatin for the indicated times and then were harvested at the end of incubation for Western blotting and RNA isolation. When indicated, cells were incubated with inhibitors of p38 MAP kinase (10 μM SB203580), ERK (10 μM U0126), or JNK (30 μM SP600125) added 1 h after LPS. LPS was added at the indicated concentrations 2 h after cisplatin addition. RAW264.7, M221, and MCF-7 cells were cultured in DMEM supplemented with 10% FBS. BxPC3 cells were cultured in RPMI supplemented with 10% FBS.

Cytokine and chemokine quantitation by ELISA. The levels of TNF-α, MCP-1, and MIP2 in cell culture supernatant were determined using an ELISA assay (Quantikine ELISA KIT, R&D System, Minneapolis, MN) according to the manufacturer’s instructions.

Western blotting. Cells were scraped from the dish and centrifuged at 10,000 g for 5 min. The cell pellet was solubilized in RIPA buffer containing protease and phosphatase inhibitor cocktails. The protein concentration was measured using the BCA protein assay reagent (Pierce, Rockford, IL). One hundred-microgram aliquots of total protein were resolved in 10% polyacrylamide gels and then transferred to a polyvinylidene difluoride membrane. The membrane was probed with phospho-specific antibodies to p38 MAPK, ERK, JNK, and Mnk1 (Cell Signaling Technologies, Beverly, MA). Antibodies for eEF-4E and eEF4E-BP1 were described previously (14, 15). Proteins were detected using enhanced chemiluminescence detection reagents.

P38 MAPK assays. p38 MAPK activity was measured by immunoprecipitation and phosphorylation of ATF-2 (p38 MAPK assay kit, Cell Signaling Technologies) as described in previous work from this laboratory (34). Briefly, cells were lysed in lysis buffer and incubated on ice for 10 min. The homogenate was centrifuged at 10,000 rpm for 5 min, and the supernatant was transferred to a fresh tube. Two hundred micrometers of supernatant protein in 200-μl volume were immunoprecipitated using immobilized p38 antibody beads. The mixture was centrifuged and washed with lysis buffer twice and kinase buffer twice. The pellet was resuspended in 50 μl kinase buffer containing 200 μM ATP and 2 μg of ATF-2 fusion protein and incubated for 30 min at 30°C. The reaction was arrested by adding SDS-sample loading buffer and then separated on a 4–12% polyacrylamide gel. Proteins were transferred onto a PVDF membrane and probed with anti-phospho ATF-2 antibody. Phospho ATF-2 was visualized using ECL reagents.

Statistical methods. All assays were performed in duplicate. The data are reported as means ± SE. Statistical significance was assessed by unpaired, two-tailed Student’s t-test for single comparison or ANOVA for multiple comparisons.

RESULTS

Synergistic interaction between endotoxin and cisplatin on TNF-α protein production by renal epithelial cells. We examined the effects of cisplatin and endotoxin, alone and in combination, on TNF-α protein production by cultured mouse kidney proximal tubule cells. Over the time course examined (up to 6 h), neither 1 μg/ml LPS nor 100 μM cisplatin resulted in a significant increase in TNF-α production (Fig. 1). However, the combination of 1 μg/ml LPS and 100 μM cisplatin resulted in early and robust production of TNF-α. Dose-response experiments indicated that increases in cisplatin over the concentration range of 10 to 100 μM in the presence of a fixed concentration of LPS resulted in progressive increases in TNF-α production (Fig. 2). The reduction in TNF-α production at 200 μM cisplatin was in association with cell toxicity (not shown). At a constant cisplatin concentration, increases in LPS over the range of 1 to 1,000 ng/ml resulted in progressive increases in TNF-α production.

Role of MAPK pathways in cisplatin-induced TNF-α production. We previously reported that p38 MAPK activation is a critical step in cisplatin-mediated TNF-α production in the kidney (34). Therefore, we examined the interactions between cisplatin and LPS on the activation of MAPK pathways. Based on the results in Fig. 2, we selected a cisplatin concentration of 100 μM and LPS concentration of 250 ng/ml for subsequent experiments. Cisplatin activated ERK, p38, and JNK kinases (Fig. 3). In contrast, LPS had no effect on the phosphorylation state of these proteins. Treatment with both LPS and cisplatin resulted in a profile of MAPK phosphorylation which was similar to that of cisplatin alone. To determine the possible role of MAPK pathways in TNF-α production, cells were treated with cisplatin and LPS along with inhibitors of ERK, p38, and JNK. TNF-α protein production was dramatically increased by the combination of cisplatin and LPS (Fig. 4) and was significantly inhibited by either ERK or p38 MAP kinase, but not JNK, inhibitors. Inhibition of both ERK and p38 MAP kinase resulted in almost complete suppression of TNF-α protein production. We previously demonstrated that the combination of cisplatin and LPS increased TNF-α mRNA (33) but that the increase was independent of MAP kinases.

Mechanism of cisplatin and LPS synergy. The previous results suggest that cisplatin increases the translation of TNF-α mRNA through pathways involving ERK and/or p38. Also of note is the finding that LPS, although inducing transcription of the TNF-α gene, did not lead to TNF-α protein production indicative of a block in TNF-α mRNA translation. Decreases in translational efficiency have been observed in animal models of sepsis, in which circulating levels of endotoxin are elevated
In studies of skeletal and cardiac muscle, sepsis interfered with translation initiation (18, 19). eIF4E is the rate-limiting component of eukaryotic translation initiation (9). Since both ERK and p38 MAP kinase pathways, acting through Mnk1, can result in the phosphorylation of eIF4E (53, 54), we examined the effects of cisplatin and LPS on the phosphorylation states of eIF4E and Mnk1. As shown in Fig. 5, basal levels of p-eIF4E and p-Mnk1 are low in TKPTS cells. Cisplatin treatment, but not LPS, increased phosphorylation of both eIF4E and Mnk1. The cisplatin-induced phosphorylation of these proteins persisted in the presence of LPS. Treatment of cells with inhibitors of MAP kinase pathways indicated that Mnk1 phosphorylation and eIF4E phosphorylation were dependent on p38 MAP kinase and, to a lesser extent, ERK activity. In the presence of both p38 MAP kinase and ERK inhibitors, cisplatin plus LPS-induced Mnk1 and eIF4E phosphorylation was completely prevented. In contrast, inhibition of JNK had no effect on Mnk1 or eIF4E phosphorylation. Overall, the effect of MAP kinase inhibitors on Mnk1 and eIF4E phosphorylation (Fig. 5) mirrored their effects on TNF-α protein production (Fig. 4). The mammalian target of rapamycin (mTOR) pathway has also been implicated in the translation initiation defect associated with sepsis (19). However, the phosphorylation of eIF4E-BP1, a substrate of mTOR (10), was not affected by cisplatin or LPS. Similarly, TNF-α protein production was not affected by inhibition of either mTOR or its upstream kinase, phosphatidylinositol 3-kinase (Fig. 6). Thus mTOR does not appear to be involved in cisplatin-induced TNF-α production.

Cell specificity of cisplatin-LPS interactions. LPS did not stimulate TNF-α production by TKPTS cells. We wished to determine whether cells which do respond to LPS also showed synergy with cisplatin. RAW264.7 murine macrophages were treated with 1 mg/ml LPS, 50 μM cisplatin, or both agents in combination. As shown in Fig. 7, macrophages produced large amounts of TNF-α in response to LPS alone. Cisplatin alone had minimal effect on TNF-α production and did not increase LPS-stimulated TNF-α production. As shown in Fig. 8, LPS alone increased activation of p38 MAPK and phosphorylation of Mnk1 and eIF4E as early as 1 h after treatment, which paralleled an increase in TNF-α production in RAW264.7 cells. Cisplatin alone increased p38 MAPK activation at a later time (6 h), but there was no additive effect of cisplatin and LPS on p38 MAPK activity. As in TKPTS cells (Fig. 5), inhibition of p38 MAPK in macrophages reduced Mnk1 and eIF4E phosphorylation (Fig. 8) and TNF-α production (not shown). Since SB203580 is a reversible inhibitor of p38 MAPK activity, ATF-2 phosphorylation in vitro was not reduced by treatment of cells with SB203580 in culture. Although cisplatin-induced TNF-α production contributes to cisplatin nephrotoxicity (35, 36), there is no evidence that cisplatin-induced TNF-α production contributes to the antitumoral activity of cisplatin. In light of the synergy between cisplatin and LPS in promoting TNF-α production in renal epithelial cells, we determined whether cispla-
tin and LPS also have synergy in certain tumor cells. MCF-7 and M221 human breast cancer cells and BxPC3 human pancreatic carcinoma cells were treated with cisplatin and LPS alone and in combination. As shown in Fig. 9, neither cisplatin nor LPS alone resulted in TNF-α production. The combination of cisplatin and LPS had no effect on TNF-α production by MCF-7 cells but increased TNF-α production by M221 and BxPC3 cells, albeit at lower levels than in renal epithelial cells (Figs. 3, 4, and 7). In MCF-7 cells, LPS did not increase the activation of p38 MAPK, Mnk1, or eIF4E (not shown), which is consistent with the blunted response in TNF-α production.

Cytokine specificity of cisplatin-LPS interaction. Earlier studies have documented that cisplatin induces the expression of several chemokines and cytokines in the kidney (35). In light of the synergy we observed between cisplatin and LPS in TNF-α production, we examined whether LPS also augments the production of other cytokines induced by cisplatin. Figure 10 shows the production of MCP-1 and MIP2 in TKPTS cells in response to cisplatin, LPS, or both in combination. When

Fig. 4. Effect of MAPK inhibitors on cisplatin- and LPS-induced TNF-α protein production in TKPTS cells. TKPTS cells were treated with 100 μM cisplatin followed by 50 ng/ml LPS in the presence or absence of MAPK inhibitors SB203580 (10 μM), U0126 (10 μM), or SP600125 (30 μM). TNF-α protein was measured in culture supernatant 6 h after LPS addition. *P < 0.001 vs. CIS+LPS; n = 4–12.

Fig. 5. Effect of MAPK inhibitors on activation of Mnk1, eIF4E, and eIF4E-BP1. TKPTS cells were treated with either saline, cisplatin (100 μM), LPS (250 ng/ml), or cisplatin followed by 250 ng/ml LPS in the presence or absence of MAPK inhibitors SB203580 (10 μM), U0126 (10 μM), or SP600125 (30 μM). Cells were harvested 6 h after LPS addition, and Western blot analysis was performed using antibodies specific to phospho-Mnk1, phospho- and total eIF4E, and eIF4E-BP1. Phosphorylation of eIF4E-BP1 is detected as a change in the molecular weight of the protein.

Fig. 6. Role of the phosphatidylinositol 3-kinase (PI3-kinase) pathway in cisplatin- and LPS-induced TNFα production in TKPTS cells. TKPTS cells were treated with either cisplatin (100 μM) or LPS (250 ng/ml) or cisplatin followed by LPS in the presence or absence of the PI3-kinase inhibitor LY294002 (50 μM) or mammalian target of rapamycin (mTOR) inhibitor (10 nM). TNF-α protein was measured in culture supernatant 6 h after LPS addition. *P < 0.001 vs. LPS or cisplatin; n = 4–6.

Fig. 7. Time course of cisplatin- and LPS-induced TNF-α production in RAW264.7 (macrophage) cells. RAW264.7 cells were treated with either 50 μM cisplatin (●) or 1 μg/ml LPS (▲) or cisplatin followed by LPS (▲) or saline (■). Culture supernatants were collected at different time intervals, and TNF-α was quantitated; n = 4–6.
The failure of LPS to activate p38, ERK, or JNK in TKPTS cells while cisplatin cells. We found, surprisingly, that LPS did not result in the role of these pathways in TNF-α production in a variety of settings (11, 12, 17, 21, 22, 24, 56). Accordingly, we examined the interactions between cisplatin and LPS on the activation of MAPK pathways and the production in macrophages (23, 28). This system, then, may be useful in defining the mechanisms responsible for the synergistic nephrotoxicity of cisplatin and LPS.

Discussion

The present study examined the interactions between two common renal insults, cisplatin and endotoxin. Zager et al. (58) recently reported that cisplatin potentiates LPS-induced TNF-α production, although the mechanisms and functional consequences were not determined. We explored the mechanisms responsible for enhanced TNF-α production using cultured mouse proximal tubule cells. We confirmed that exposure of renal tubular epithelial cells to cisplatin and LPS resulted in marked synergy in the production of TNF-α (Figs. 1 and 2). Although LPS by itself induced no TNF-α protein production in TKPTS cells, several observations indicate that these cells are capable of responding to LPS. First, LPS resulted in a large but transient increase in TNF-α mRNA in TKPTS cells (33). Second, TKPTS cells express TLR4 (not shown), the receptor for LPS (32). Finally, in the presence of cisplatin, LPS produced a dose-related increase in TNF-α protein production (Fig. 2). Moreover, the concentrations of LPS required for this effect (∼10 ng/ml) are similar to those which induce TNF-α production in macrophages (23, 28). This system, then, may be useful in defining the mechanisms responsible for the synergistic nephrotoxicity of cisplatin and LPS.

MAP kinases are involved in the regulation of TNF-α production in a variety of settings (11, 12, 17, 21, 22, 24, 56). Similarly, cisplatin activates p38, ERK, and JNK/SAPK in various tissues (30, 31, 39, 51), including the kidney (2, 27, 34). Accordingly, we examined the interactions between cisplatin and LPS on the activation of MAPK pathways and the role of these pathways in TNF-α production by renal epithelial cells. We found, surprisingly, that LPS did not result in the activation of p38, ERK, or JNK in TKPTS cells while cisplatin activated all three kinase pathways. The failure of LPS to activate any of the MAPK pathways in renal epithelial cells contrasts with the activation of these pathways by LPS in leukocytes (26, 44). As will be discussed below, the differential ability of cisplatin and LPS to activate p38 MAPK is critical to their synergy.

TNF-α gene transcription is controlled by multiple enhancer elements, including three NF-κB sites, a cAMP response element, an AP-1 binding site, and an Egr site (46, 55). LPS stimulation of TNF-α gene transcription in neutrophils, monocytes, and macrophages is highly dependent on MAPK activation (26, 38, 44). However, the ability of LPS to increase TNF-α mRNA content in TKPTS cells in the absence of MAPK activation along with the lack of effect of MAPK measured 6 h after treatment, cisplatin alone had no effect on either MCP-1 or MIP2 production whereas LPS alone induced a significant increase in the production of both chemokines. However, the rates of production in the presence of both cisplatin and LPS were no greater than with LPS alone. Thus synergistic stimulation of cytokine production is not a universal finding and may reflect cytokine-specific differences in their translational regulation.

Discussion

The present study examined the interactions between two common renal insults, cisplatin and endotoxin. Zager et al. (58) recently reported that cisplatin potentiates LPS-induced TNF-α production, although the mechanisms and functional consequences were not determined. We explored the mechanisms responsible for enhanced TNF-α production using cultured mouse proximal tubule cells. We confirmed that exposure of renal tubular epithelial cells to cisplatin and LPS resulted in marked synergy in the production of TNF-α (Figs. 1 and 2). Although LPS by itself induced no TNF-α protein production in TKPTS cells, several observations indicate that these cells are capable of responding to LPS. First, LPS resulted in a large but transient increase in TNF-α mRNA in TKPTS cells (33). Second, TKPTS cells express TLR4 (not shown), the receptor for LPS (32). Finally, in the presence of cisplatin, LPS produced a dose-related increase in TNF-α protein production (Fig. 2). Moreover, the concentrations of LPS required for this effect (∼10 ng/ml) are similar to those which induce TNF-α production in macrophages (23, 28). This system, then, may be useful in defining the mechanisms responsible for the synergistic nephrotoxicity of cisplatin and LPS.

MAP kinases are involved in the regulation of TNF-α production in a variety of settings (11, 12, 17, 21, 22, 24, 56). Similarly, cisplatin activates p38, ERK, and JNK/SAPK in various tissues (30, 31, 39, 51), including the kidney (2, 27, 34). Accordingly, we examined the interactions between cisplatin and LPS on the activation of MAPK pathways and the role of these pathways in TNF-α production by renal epithelial cells. We found, surprisingly, that LPS did not result in the activation of p38, ERK, or JNK in TKPTS cells while cisplatin activated all three kinase pathways. The failure of LPS to activate any of the MAPK pathways in renal epithelial cells contrasts with the activation of these pathways by LPS in leukocytes (26, 44). As will be discussed below, the differential ability of cisplatin and LPS to activate p38 MAPK is critical to their synergy.

TNF-α gene transcription is controlled by multiple enhancer elements, including three NF-κB sites, a cAMP response element, an AP-1 binding site, and an Egr site (46, 55). LPS stimulation of TNF-α gene transcription in neutrophils, monocytes, and macrophages is highly dependent on MAPK activation (26, 38, 44). However, the ability of LPS to increase TNF-α mRNA content in TKPTS cells in the absence of MAPK activation along with the lack of effect of MAPK measured 6 h after treatment, cisplatin alone had no effect on either MCP-1 or MIP2 production whereas LPS alone induced a significant increase in the production of both chemokines. However, the rates of production in the presence of both cisplatin and LPS were no greater than with LPS alone. Thus synergistic stimulation of cytokine production is not a universal finding and may reflect cytokine-specific differences in their translational regulation.
inhibitors on TNF-α mRNA levels (33) suggest that TNF-α gene transcription in TKPTS cells depends primarily on NF-κB rather than MAP kinases. Although the effect of LPS and cisplatin on TNF-α mRNA levels was independent of MAPK activation, TNF-α mRNA translation was dependent on p38 MAPK and, to a lesser extent, ERK. Inhibition of both kinases completely abolished TNF-α production in response to LPS and cisplatin (Fig. 4). A role for p38 MAPK in TNF-α activation, TNF-α epithelial cells (24, 34). Although JNK activation was required for LPS-stimulated TNF-α production in macrophages (44), inhibition of JNK increased TNF-α production by TKPTS cells. The inability of LPS to activate p38 MAPK in TKPTS cells likely accounts for the synergy noted between LPS and cisplatin, which does activate p38 MAPK. Treatment of RAW264.7 murine macrophages with LPS resulted in activation of p38 MAPK (Fig. 8) and production of large amounts of TNF-α protein (Fig. 7). Moreover, in these cells there was no added effect of cisplatin on TNF-α production. The disparate role of MAPKs in TNF-α gene transcription and mRNA translation in renal epithelial cells vs. leukocytes underscores the cell specificity of the regulation of TNF-α production (47). The lack of significant synergy between cisplatin and LPS in certain tumor cell lines (Fig. 9) is yet another example of cell specificity. Based on our findings, strategies to enhance the tumoricidal activity of cisplatin using TLR4 agonists would be expected to increase the toxicity of cisplatin without, perhaps, an increase in tumor cell death.

The failure of LPS, by itself, to stimulate TNF-α protein production while stimulating TNF-α mRNA production prompted us to examine the effects of LPS and cisplatin on the translational apparatus. Sepsis has been associated with defects in mRNA translation and, more specifically, with translation initiation (18, 19, 50). An important mechanism to regulate the initiation process is the modulation of the availability of eIF4E to form an active eIF4F complex (4, 9). Two determinants of eIF4E availability are the amount of eIF4E present within the cell and the extent of association of eIF4E with a family of translational repressors, the eIF4E-binding proteins (4E-BPs). Phosphorylation of 4E-BP1 inhibits its association with eIF4E, thereby increasing eIF4E availability for translation initiation (8). In addition, phosphorylation of eIF4E itself is generally associated with increased rates of protein translation, although the exact mechanism remains unclear (40). Our results indicate that cisplatin treatment, but not LPS, increases the phosphorylation of eIF4E. The eIF4E phosphorylation was dependent on p38 MAPK and likely mediated through activation of Mnk1. In contrast, the levels of total eIF4E (Fig. 5) and the phosphorylation of eIF4E-BP1 were not affected by cisplatin, LPS, or MAPK inhibitors. These results are consistent with the view that cisplatin increases translation initiation of TNF-α mRNA via p38 MAPK-dependent phosphorylation of eIF4E rather than by increasing eIF4E abundance or decreasing its association with eIF4E-BP1.

Our view of the mechanism underlying the synergy between LPS and cisplatin, based on the above findings, is that LPS primes renal epithelial cells to produce TNF-α by increasing TNF-α gene transcription in a MAPK-independent manner. Cisplatin, on the other hand, has only a modest effect on TNF-α gene transcription but dramatically increases TNF-α mRNA translation through activation of p38 MAPK and ERK. Either agent alone results in little TNF-α production: LPS due to a lack of mRNA translation and cisplatin due to a lack of gene transcription. When both are present, however, large amounts of TNF-α are produced, resulting in acute renal failure and, perhaps, contributing to extrarenal toxicity. This scenario also predicts that other kidney conditions associated with p38 MAPK activation should exhibit synergy with endotoxin. Indeed, p38 MAPK is activated in renal ischemic injury (24, 29, 57), aminoglycoside toxicity (52), and urinary tract obstruction (43), and all of these types of injury exhibit synergy with endotoxin (58–60).

Finally, it is tempting to speculate, based on the present results, that coexisting infections might influence an individual’s susceptibility to developing cisplatin nephrotoxicity. Similarly, acquired or genetic differences in toll-like receptor signaling or downstream effector pathways, such as TNF-α production and action, might also determine the risk of cisplatin nephrotoxicity. If so, knowledge of these determinants could be used to guide the clinical use of cisplatin.

**GRANTS**

This study was supported by the National Institute of Diabetes and Digestive and Kidney Diseases (RO1-DK-063120 to W. B. Reeves) and the Veterans Affairs Medical Research Service. G. Ramesh was a fellow of the National Kidney Foundation when this work was performed.

**REFERENCES**

Effects of Endotoxin and Cisplatin on TNF-α Production


