# CHAPTER V

# DISCUSSION AND CONCLUSION

## Unilateral Ureteral Obstruction and Immune Cells

Unilateral ureteral obstruction (UUO) is a condition in which the lymphocytes play an important role in renal pathology.[152,153] It has been demonstrated that UUO induces the infiltration of macrophages and lymphocytes (both suppressor and cytotoxic T cells) in renal tissue in both medulla and cortex.[12] These cells could produce many major cytokines, such as TNF, resulting in the main pathology of kidney, namely the inflammation and fibrosis of renal tissue. If the obstruction continues and remains unsolved, this causes apoptosis of renal tissue and, in turn, chronic renal failure.[155,156]

Besides the investigation of lymphocyte infiltration and causes of renal tubular apoptosis, there are no data studying of number of circulating lymphocytes under UUO condition. Therefore, the present study shows the first evidence regard to this number as well as its percentage of cell death and apoptosis. In present study, the number of circulating lymphocyte seemed to decline as compared to the control group (no operation). This may explain by the fact that the circulating lymphocytes had infiltrated into the obstructive area during UUO condition (a significant reduction was observed on day 4). However, the lower levels of the cells during UUO were comparable to those of sham operative animals. After an operation, the body starts to repair the incision.[148-150] During healing process, various kinds of leukocyte take care of phagocytosis.[148-150] This would result in a slight reduction of circulating lymphocyte in the sham groups.

**Unilateral Ureteral Ostruction, Angiotensin System and Apoptosis**

It has been shown that UUO causes increased levels of angiotensin II (Ang II) in both renal tissue and plasma.[14-16] This is a major mechanism that induces renal tubular cell apoptosis.[174] In other cell types, Ang II also causes apoptosis.[176] For example, the *in vitro* study of ventricular cardiomyocytes showed that Ang II increased apoptosis of the cells.[173]

The mechanisms by which Ang II induces this cell apoptosis are an activation of caspase-3[176] and of Bax protein (an inducer of apoptosis).[177,178] Moreover, Ang II also stimulates G-protein coupled receptor in cardiomyocytes.[179] Sustained or excessive activation of either Gq-or Gs-signaling pathways (MAP kinase) results in apoptosis of cardiomyocytes both *in vitro* and *in vivo*.[179] In addition, UUO increases p53 protein (an inducer of Bax transcription) that could induce the local Ang II production in ventricular myocytes and then trigger apoptosis.[180]

The recent study also showed that Ang II mediates alveolar epithelial cell apoptosis via angiotensin receptor subtype 1 (AT1).[181] Furthermore, the *in vitro* study of human endothelial cell demonstrated that Ang II induces apoptosis signaling via MAP kinase phosphatase-3 (MKP-3)-dependent dephosphorylation of ERK1/2, which in turn leads to the degradation of Bcl-2 (an antiapoptotic protein).[182] When the inhibition of angiotensin system, either by $AT_1$ receptor antagonist (ARA) or by angiotensin converting enzyme inhibitor (ACEI), was performed the apoptosis of the cells was normalized.[177,178,180,183]

No such experiments performed an *in vitro* study of direct effects of Ang II on apoptosis of isolated circulating lymphocytes. Besides that, the present *in vivo* study sought to investigate the influence of an endogenously higher Ang II level during UUO on circulating lymphocyte apoptosis. The discussion related to this regard is followed.

**Circulating Lymphocyte Apoptosis and Angiotensin System**

The regulation of lymphocyte survival is of fundamental importance to the functioning of the immune system as a whole.[184] In the past several years, it has become apparent that the control of apoptosis of lymphocytes is a complex and multifaceted process.[184] There is an expanding number of cell surface signaling molecules, on both B and T cells, that an ligand binding could affect the apoptotic threshold. These receptors signal through multiple distinct and likely interacting intracellular pathways. This multilayered web includes the tumor necrosis factor receptor (TNFR) family.[184] The family members expressed on lymphocytes are $TNFR_1$, $TNER_2$, Fas, CD40, CD30, CD27, and 4-1BB.[185] Furthermore, another set of cell surface molecules, unrelated to the TNFR family, whose role in T-lymphocyte apoptotic regulation is being increasingly recognized, consists of the CD28 and CTLA4 receptors.[186]

Classically, Ang II is recognized as a relatively potent vasoconstrictor.[13] It has been demonstrated that Ang II also plays a role as an immune costimulator.[187] The expression of $AT_1$ receptor has been found on the surface of lymphocytes and spleenocytes.[187] Moreover, lymphocytes (T-cells) also express angiotensin converting enzyme that is the highest level, approximatedly 28 times more per cell than monocytes.[188] No

activity of the enzyme was detected in B lymphocytes. In addition, lymphocytes contain a protein kinase activity that catalyzes the phosphorylation of both endogenous and exogenous substrates on tyrosine residues.[189] Kinetic analyses indicate that angiotensin I could serve as a substrate for tyrosine protein kinase in T lymphocytes.[189] Therefore, lymphocytes could be undergone apoptosis not only upon by Ang II ligand binding but also by increasing endogenous Ang II production.

In the present results, UUO caused circulating lymphocyte apoptosis in both 1-day and 7-day duration. After blockage of angiotensin system either with ACEI or with ARA, the lymphocyte apoptotic index significantly reduced to near their respective sham groups. This result agrees with the study by Deas, O. et al,[190] that ACEI inhibits Fas-induced apoptosis in human activated T cell.[191] However, the reduction of heightened apoptotic index occurred at 7-day period of UUO. The one-day treatments showed no effects on cell apoptosis. This suggests that the process of decreased circulating lymphocyte apoptosis is a time-dependent inhibition of angiotensin system. Therefore, UUO induces circulating lymphocyte apoptosis by the mechanism mediated via angiotensin system.

Regards to percentage of cell death, the present data show that the ACEI treatment, but not ARA, could significantly attenuate these levels in both 1-day and 7-day UUO animals. The exact mechanisms to explain this result remain to be investigated. However, many studies have proposed various supporting explanations[190,192-198] Angiotensin converting enzyme inhibitor could increase antioxidant activity[192-194], reduce metalloproteases,[195,196] and inhibit Fas-induced apoptosis.[190] Some of these

properties may relate to the presence of thio groups in ACEI structure and are independent of its effect on the renin angiotensin system.[197,198]

All together, the results in the present study are the first evidence to show that, during UUO, the angiotensin system plays a pivotal role in circulating lymphocyte apoptosis. The longer time to block this system could attenuate the induction of apoptotic lymphocyte cells. This may result in an improvement of immune defense mechanism during UUO.

The proposed mechanisms that angiotensin II induces apoptosis of circulating lymphocyte are shown in Figure 24.

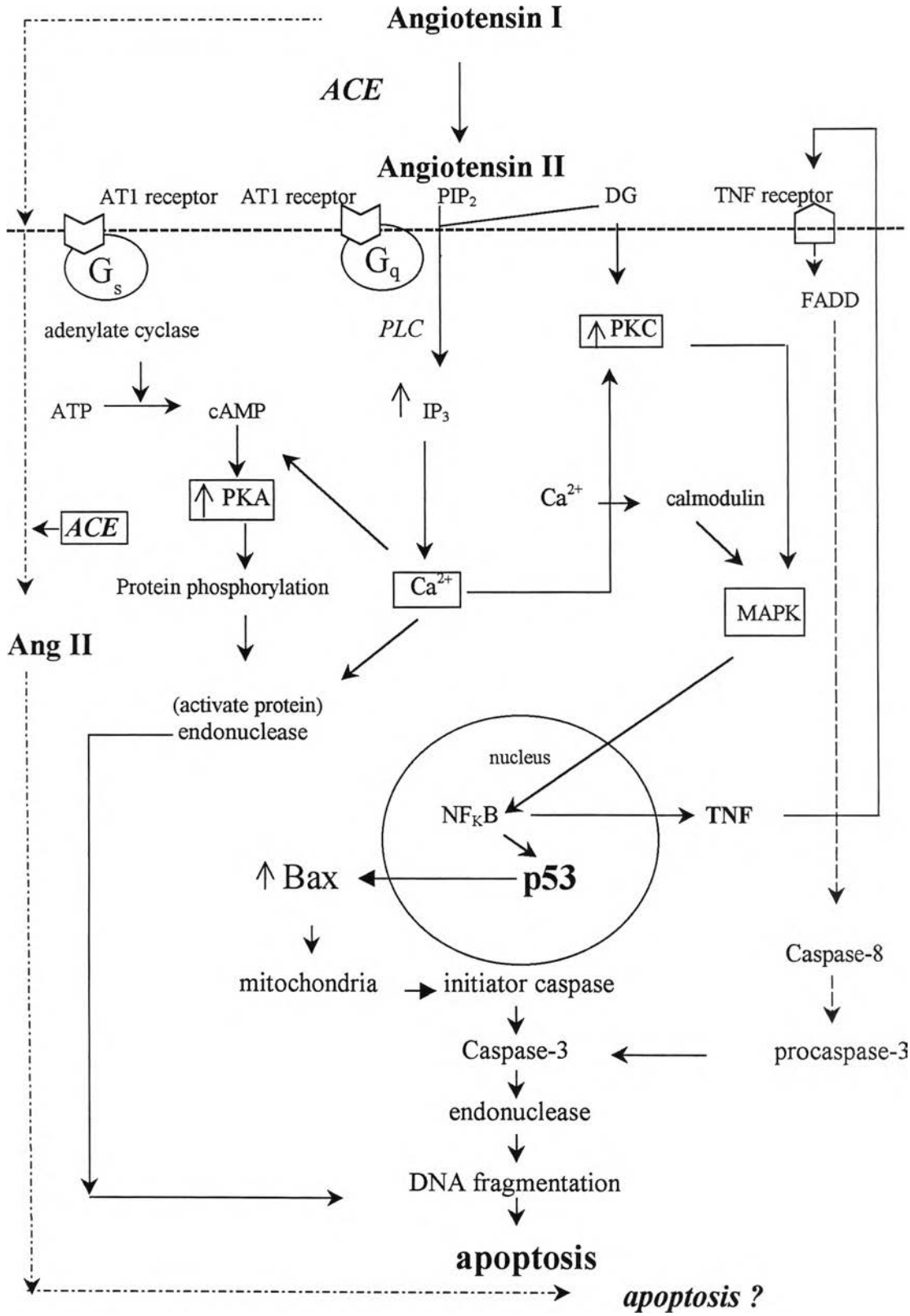


**Figure 24** The proposed mechanisms that angiotensin II induces apoptosis of circulating lymphocyte

**Note:** ----- refers to cell membrane of lymphocyte