CHAPTER V

DISCUSSIONS

Humans infected with pathogenic *Leptospira* demonstrate a diverse array of clinical manifestations, which is from asymptomatic to severe or fatal diseases. Although several leptospiral components have been identified and suggested to be virulence factors, the mechanism of pathogenesis of this infectious disease is still not clearly defined. The ability of *Leptospira* to cause different degrees of symptoms may involve multiple factors. Moreover, pathogenic *Leptospira* can be divided into more than 200 serovars and whether different serovars can cause disease with different manifestations, is still unclear.

Most studies detected cytokines in peripheral blood mononuclear cells from human or animals infected by *Leptospira* or in cell lines stimulated with *Leptospira* or glycoproteins/outer membrane proteins. The purpose of this study is to compare the mRNA expression of LipL32, TNF-α, IL-10, TGF-β and IP-10 in kidneys and livers infected with *Leptospira interrogans* serovar Pyrogenes which are pathogenic species compared with uninfected hamsters. Infected hamsters prepared by injected intraperitoneal with 10^8 *Leptospira interrogans* serovar Pyrogenes and collected kidney and liver tissues on day 3, 5 and 7 postinfection. Four hamsters were used for each timepoint. Four uninfected hamsters were used as a control group. Pathological changes in kidneys, livers were detected since day 3 after infection. In kidneys, tubular cell injury or tubular necrosis was observed. Swelling and congestion of hepatocytes occurred in infected livers.

16S rRNA expression was investigated to demosntrate the presence of *Leptospira* and the result showed that 16SrRNA could be detected in all infected kidneys and infected livers. The data suggested that leptospires could be found in kidneys and livers on day 3 (or before) post-infection. It has been shown that *Leptospira* could be detected in kidney tissues by realtime PCR since the first day

of infection (194).

Several *Leptospira* components such as lipopolysaccharide, outer membrane proteins, and various secretory proteins have been studied (4-11). Among OMPs reported, as mentioned earlier, LipL32 is the most widely studied as a candidate for diagnostic tool and vaccine development. In addition, it has been suggested to involve in disease pathogenesis. LipL32 expression in kidneys has been demonstrated (30). The liver is another organ where pathologies were observed in leptospirosis. In this study, LipL32 expression was observed in both kidney and liver tissues of infected hamsters (Figure 7 and 8) which confirmed that this protein was expressed *in vivo*. Its expression could be detected in all day 3, 5 and 7 samples. The relative level of LipL32 expression was similar in all samples even when SYBR-real time PCR was performed.

Several investigators suggested that immune response may be involved in pathologies induction. It has been demonstrated that colonization of leptospires in proximal tubule epithelial cells resulting in acute renal failure and tubulointerstitial nephritis. Praditpornsilpa K et al. demonstrated that immunomodulation by rapamicin alleviated kidney and pulmonary injuries in hamsters infected with *Leptospira interrogans* serovar Pyrogenes (23). Various cytokine productions or gene expressions in the response to *Leptospira*, leptospiral glycoproteins or outer membrane proteins have been demonstrated as described earlier. LipL32 has been shown to induce cytokines induction in mouse proximal tubule cells (18, 26, 195). In addition, LipL32 is a prominent immunogen during human leptospirosis (196) and may be related with cytokine induction (26). Most studied investigated cytokine induction by LipL32 *in vitro*. In this study, we investigated TNF-α, IL-10, TGF-β and IP-10 mRNA expression in kidneys and livers infected with pathogenic *Leptospira*. The results of this experiment may demonstrate the correlation between LipL32 and cytokine expression in these organs.

TNF-α expression was detected in kidneys and livers of infected hamsters. However, it is difficult to interpret whether *Leptospira* infection enhanced TNF-α expression in kidneys since its expression could also be detected in kidneys of uninfected hamsters (Figure 12A). Expression of TNF-α in uninfected livers was undetectable (Figure 12B). In infected hamsters, TNF-α expression

could be demonstrated in livers. On day 3, TNF-α expression could be detected in 3 out of 4 hamsters. On day 5, 1 out of 4 hamsters demonstrated TNF-α expression. On day 7, 2 out of 4 hamsters demonstrated TNF-α expression (Figure 14). Similar to TNF-α, TGF-β expression could be detected both in kidneys from uninfected and infected hamsters (Figure 15A and Figure 16). TGF-β expression was undetectable in livers of uninfected livers (Figure 15B) whereas its expression could be demonstrated in infected hamsters (Figure 17). TGF-β expression could be demonstrated in 4 out of 4, 3 out of 4 and 4 out of 4 infected livers on days 3, 5 and 7 post-infection, respectively. These suggested that *Leptospira* infection enhanced TNF-α and TGF-β expression in livers of infected hamsters.

As mentioned earlier that IL-10 was studied in order to investigate whether there is the difference between IL-10 and another anti-inflammatory cytokine, TGF-β expression in infected kidneys and livers. IL-10 expression was undetectable in uninfected kidneys and livers (Figure 18). Its expression could be observed in infected kidneys (Figure 19). Interestingly, whereas TNF-α and TGF-β expression was enhanced in infected livers (Figure 14 and Figure 17), the IL-10 expression was undetectable in this organ (Figure 20).

Vernal-Pauillac et al. (15) reported that TGF-β expression could be detected before IL-10 expression in blood of infected hamsters. TGF-β expression was found as early 8 hours postinfection whereas IL-10 was detected in day 3 postinfection. This report supported our result which the density of TGF-β expression was relatively higher than that of IL-10 expression in infected kidneys.

De Fost et al. (29) reported that level of IP-10 in blood of leptospirosis patients was higher than healthy. In this study, we found that, similar to TNF-α and TGF-β, IP-10 expression could be detected in kidneys but not in livers of uninfected hamsters (Figures 21A and 21B). The relative level of expression in kidneys compared with HPRT was higher in infected than in uninfected hamsters (Figures 21 and 22). Real-time PCR was performed to quantitate the level of expression of IP-10 in infected over uninfected kidneys. As shown in Figure 25, the relative level was higher in infected than uninfected kidneys. The average level of IP-10 expression in infected kidneys was increase

from day 3 to day 7 postinfection. Similar to IL-10, IP-10 expression was undetectable in livers of infected hamsters.

Chemokines are involved in attraction and retention of lymphocytes in infected tissues. Interferon-gamma inducible protein-10 kDa (IP-10) or CXCL-10 is a CXC chemokine which is a T cell chemoattractant. Expression of IP-10 has been found among patients suffering from diseases associated with a type 1 immune response, including tuberculosis, scrub typhus, *Helicobacter pylori* gastritis and chronic viral hepatitis. It has been reported that *Leptospira* activated T cell expansion. Plasma IP-10 and Mig (monokine induced by IFN-γ) were higher in patients with definite or possible leptospirosis than in healthy donors. Other chemokines such as monocyte chemotactic protein-1, neutrophil-chemoattractant chemokines (CXCL1/KC) and CKCL2/MIP-2 were induced by leptospiral outer membrane protein (18, 26, 195). Our study demonstrated in kidneys that IP-10 expression was relatively high when compared with other cytokines studied which suggested that the recruitment of T lymphocytes to infected kidneys was involved in immune response or pathogenesis in leptospirosis in this organ.

It has been shown that IP-10 expression was induced in liver tissues of patients with chronic hepatitis C virus (HCV) infection and its expression correlated with liver inflammation(197, 198) However, in our study, the expression of IP-10 was undetectable in livers from hamsters infected with *Leptospira* which suggested that IP-10 induction in liver tissues induced by *Leptospira* and HCV may be different.

The results obtained from cytokine expression studies suggested that cytokine expression patterns in kidneys and livers of infected hamsters were different. *Leptospira* infection enhanced TNF-α and TGF-β expression in livers whereas IL-10 and IP-10 induction could not be observed in this organ. However, in infected kidneys, induction of IL-10 and IP10 expression was obviously demonstrated. Although, both kidneys and livers contain cells responsible for IP-10 and IL-10 secretion, the expression of these two cytokines induced by *Leptospira* in these two organs were different. LipL32 expression could be detected in both kidney and livers. This suggests that LipL32

may not play role in IL-10 or IP-10 induction in these two organs.

Liver enriches of innate immune cells such as macrophage and is involved in removal of waste molecules and immunologic elimination of microorganisms by liver endothelial call and kupffer cells. Kupffer cells are abundant in livers and act as the macrophage. It can promote stellate cell activation via the production of cytokines/growth factors such as TGF-β (199). In this study, IL-10 and IP-10 expressions in infected livers were undetectable. Kuffer cells may induce production of TGF- β which acts as an anti-inflammatory cytokine instead of IL-10. In addition, Kupffer cells may remove *Leptospira* from livers effectively before the IL-10 and IP-10 expression were induced.