CHAPTER I

BACKGROUND

Leptospirosis is a worldwide zoonosis caused by spirochetes of the genus *Leptospira*. It has been identified as a re-emerging infectious disease in rural and urban environment in both industrialized and developing countries. Leptospirosis is transmitted by direct contact with urine from infected animals or indirect contact with contaminated water. Hematogenous dissemination of *Leptospira* throughout the infected host can result in a wide range of clinical manifestations. Clinical symptoms of leptospirosis vary from subclinical infection to variety of adverse effects. In severe cases, pulmonary hemorrhage, hepatic and renal dysfunction or multiorgan failure can occur and lead to fatality in a short time if not treated (1-3).

Molecular mechanisms underlying leptospirosis pathogenesis are not clearly elucidated. Several virulence factors such as lipopolysaccharide, outer membrane proteins (OMPs), and various secretory proteins have been studied (4-11). Various OMPs have been identified. Most studies focus on the expression of OMPs *in vivo* and *in vitro*, the conservation among pathogenic *Leptospira*, and the potential to be candidates for proper antigens or vaccines in diagnostic tool or vaccine develoment. Examples of *Leptoispira* OMPs reported are OMPL1, LipL21, LipL32, LipL36, LipL41 and LipL48. LipL32 is the most abundant and most widely studied protein.

Pathologies observed in Leptospirosis may be induced directly by *Leptospira* virulence factors. However, immune response to leptospires has been increasingly explored since it may be involved in leptospirosis pathogenesis. Various studies have demonstrated roles of cytokine induction in immune response to leptospires. *Leptospira borgpetersinii* serovar Hardjo induced IFN-γ production by CD4+T cell and CD8+T cells and *Leptospira interrogans* serovar Rachmati induced IFN-γ, IL-12p40, and TNF-α in human whole blood (12, 13). These data suggested that leptospires induced Th1 cytokine response. Campet et al. supported the role of T cells in immune response to

Leptospira by using lived leptospires to stimulate peripheral blood mononuclear cells. They found that when high number of leptospires was used, majority of expanding cells were $\gamma\delta^+$ T cells. However, low number of leptospires induced significant $\alpha\beta^+$ T cell expansion instead (14).

Expression of IL-4, IL-10, IL-12p40, TNF-α, IFN-γ, and TGF-β in peripheral blood mononuclear cells of hamsters infected with *L. interrogans* serovar Icterohaemorrhagiae were shown using real-time PCR assay (15). The increase in TNF-α, IFN-γ, and IL-12 expression could be detected since a few hours postinfection; however, and levels of IL-4 and IL-10, anti-inflammatory cytokines were prominent in delayed samples from 1 to 4 days postinfection.

Immune responses to components of leptospires have also been demonstrated. Glycolipoprotein of *Leptospira interrogans* serovar Copenhagen induced IL-6, IL-10, and TNF-α production in peripheral blood mononuclear cells (16, 17). Outer membrane protein of *Leptospira interrogans* serovar Shermani induced NF-KB activation and expression of nitric oxide, MCP-1, and TNF-α in medullary thick ascending limb cells (18). These data suggested that cytokine production may be involved in tuberlointerstitial nephritis caused by leptospirosis. Tian et al. (19) demonstrated that outer membrane protein of *Leptospira santarosai* serovar Shermani increased type I and type IV collagens and TGF-β in proximal tubular cells HK-2 cells and suggested that *Leptospira* may cause renal failure by enhancing extracellular matrix synthesis.

Study of immune response in Leptospirosis will not only provide information for proper vaccine development but also for further elucidating mechanisms of pathogenesis. An improved understanding of host immune response in leptospirosis may lead to development of more effective treatment and prevention of the disease. Most reports on cytokine production in response to leptospires were done using peripheral blood mononuclear cells from infected animals or *in vitro* stimulation of certain cell lines with *Leptospira* or *Leptospira* components. In this study, we demonstrated the presence of cytokine expreesion in organs where the pathologies occur.

The animal model used in this study was Golden Syrian hamsters because they demonstrate

manifestations of the disease similar to those of severe human disease including interstitial nephritis, hemorrhage, and jaundice (20-23). A *Leptospira* isolate, *Leptospira interrogans* serovars Pyrogenes was used in this study because it was one of serovars commonly found in Thailand and Muensoongnoen J. et al. (24) reported that this isolate induced pathologies in hamsters. Hamsters were injected intraperitoneally with *Leptospira interrogans* serovars Pyrogenes and were sacrificed on days 3, 5 and 7 postinfection since it has been shown in previous report (24) and in our pilot study using this isolate that hamsters were sick or died around days 5-7. Kidney and liver tissues were collected for RNA extraction.

Although both kideys and livers are organs commonly affected in Leptospirosis, most studies on mechanisms of pathogensis were done using kidney cells or kidney tissues. In this study, we investigated cytokine gene expression in both kidney and liver tissues. TNF-α, IL-10, TGF-β and IP-10 were selected for investigation since their involvement in leptospirosis has been reported.

TNF-α acts as a proinflammatory cytokine. It was released in response to lipopolysaccharide, other bacterial products and involved in systeinic inflammation. Several reports suggested that *Leptospira* or *Leptospira* components induced TNF-α induction in various cells such as human whole blood (13) peripheral blood mononuclear cells (16, 17, 25), medullary thick ascending limb cells (18). In addition, recombinant LipL32 induced expression of TNF-α in proximal tubule cells (26). In *in vivo* experiment, *L. interrogans* induced TNF- α induction in blood of infected hamsters. Athanazio et al. (27) reported that renal inflammation was not observed in TNFR knockout mice infected by *Leptospira interrogans* serovar Copenhageni. In addition, TNF-α has been observed in leptospirosis patient, and correlated between high levels of TNF-α and the worsening of symptoms (28). For those reasons, TNF-α was a pro-inflammatory cytokine chosen to be investigated in this study.

IL-10 and TGF-β act as the anti-inflammatory cytokines. TGF-β inhibits T cell proliferation and effectors function, B cell proliferation and IgA production, macrophage activity. IL-10 functions to down-regulate the expression of Th1 cytokines, MHC class II Ags, and costimulatory molecules

on macrophages. In addition, IL-10 inhibits synthesis of pro inflammatory cytokines. Several reports suggested that *Leptospira* or *Leptospira* components induced IL-10 and TGF-β induction (15, 16, 19). Although they both regulate immune response, as mentioned above, the mechanisms of these two cytokines in regulation of immune response are different. Their expressions were detected in blood of infected hamsters. However, TGF-β expression was increased since 8 hours post infection whereas IL-10 expression was increased on day 1 post infection (15). This study investigated both IL-10 and TGF-β expression to demonstrate whether there is the difference between these two anti-inflammatory cytokines in kidneys and livers of *Leptospira* infected hamsters.

Beside pro- and anti-inflammatory cytokines, we were also interested in chemokine expression. IP-10 was a chemokine investigated in this study. IP-10 has chemotactic effect on activated T lymphocytes, monocytes and NK cells. The induction of other chemokines has been reported. *Leptospira* or its components induced expression of MCP-1 and RANTES which are monocyte-chemotactic chemokines (18, 26). As mentioned above, *Leptospira* induced T cell expansion and IFN-γ production (14). In addition, it has been shownt that the level of IP-10, a T cell chemokine, increased in leptospirosis patients (29). We investigastged whether this T cell chemokine expression was induced in kidneys and livers by *Leptospira* infection.

In addition to cytokine gene experssion, the expression of LipL32 in kidneys and livers were also demonstratreted in this study. LipL32 is a 32-kDa lipoprotein designated LipL32. It is one of the most studied leptospiral proteins since it is thought to be important during human pathogenesis. It could be detected both *in vitro* and *in vivo* (5, 30) and its expression conserved among pathogenic species (31, 32). Immunohistochemistry of infected hamster kidney demonstrated intense LipL32 reactivity in proximal tubule cells and the interstitium which suggested that this protein may be involved in tubulo-interstitial nephritis (30). In addition, LipL32 is a prominent immunogen during human leptospirosis and may be related with cytokine induction (26). Yang et al. (26) reported that recombinant LipL32 induced the expression of MCP-1, iNOS in proximal tubule cells. It triggered an inflammatory response in mouse renal proximal tubule cells through a mechanism involving nuclear factor-KB and Toll-like receptor-2 (26, 33). These evidences make LipL32 an attractive protein for

further studies on diagsnostic tool and vaccine development and its role in pathogenesis. There are several reports demonstrating that LipL32 was a good antigen for antibody detection (31, 34-36). For vaccine development, LipL32 DNA vaccine induced protection in an animal model (37). There was no report on LipL32 expession and its involvement in cytokine production in livers. This study investigated LipL32 expression in both kidneys and livers.

Objectives

The proposes of this research are to demonstrate the mRNA expression of LipL32, TNF-α, TGF-β, IL-10 and IP-10 in kidneys and livers of hamsters infected with pathogenic *Leptospira* compared with non-infected hamsters.