CHAPTER V

DISCUSSION

SDS-PAGE protein patterns of three serovars of *L. interrogans* and one *L. biflexa* serovar patoc revealed a number of common protein bands, the overall profiles among three pathogenic and one non-pathogenic leptospires were different. This is in accordance with Chapman's report demonstrating the SDS protein pattern in three *L. interrogans* serovar, one *L. biflexa* serovar patoc and *Leptonema illini* (25). Chapman *et al*. (27) demonstrated minor differences among three *L.interrogans* serovar hardjo isolated from Australia, New Zealand, and the type strain Hardjoprajitno. In the present study, a complex pattern of protein bands with molecular weights ranging from 25 to 90 kd was readily discernible for each serovar. These results are similar to those reported by others (25, 27).

Immunoblotting of four leptospiral sonicated antigens against homologous and heterologous rabbit antisera indicated that there were some specific and numerous cross-reactive antigenic bands. The number of reactive bands was more evident in homologous systems but no serovar-specific reactive band was observed. Specific antigens of non-pathogenic *L.biflexa* serovar patoc were found on antigenic bands Mw of 52 and 46 kd. Some specific pathogenic bands were revealed on all *interrogans* tested indicating a close antigenic relationship within species.

The specific pathogenic antigens of leptospires were 45, 41, and 30 kd. A 32 kd band seemed to be specific but it showed cross-reaction with rabbit anti-*L.biflexa* serovar patoc antiserum. This is different from Niikura's report of a 32 kd antigen demonstrable in a virulent clone extract of *L. interrogans* serovar copenhageni, strain Shibaura, by westernblotting utilizing anti-virulent clone but not anti-avirulent clone antisera (26). This 32 kd antigen was found to be thermostable and not to be a protein antigen.

However, our study in immunoblotting with polyclonal rabbit antisera prepared against whole leptospires revealed not only specific antigenic bands but also common antigens in all leptospires tested. There were: a 60 kd protein associated with all serovars and doublet bands of 33-34 kd or 32-33 kd on *L. biflexa* serovar patoc or *L. interrogans* respectively. In a similar experiment (25), Chapman et al. showed a common 34-35 kd doublet bands on serovar patoc and *Leptonema illini*, and a common 34.5-35 kd on *L.interrogans*. According to these authors, the 35 kd band was a flagellar protein and also a component of the genus-specific antigen produced by ethanol fractionation. It is possible that these common proteins may be similar antigens with some variation in electorphoretic mobility.

By immunoblotting, Kelson *et al*. (28) demonstrated a major protein doublet of 35-36 kd and 33-34 kd on flagellae of *L.biflexa* serovar patoc and *L. interrogans* (and *L. illini*) respectively whereas Jost *et al*. (29) found a 35 kd protein band

to be a species-specific antigen in the outer envelopes of pathogenic leptospires. On the other hand, Sakamoto (53) showed a leptospiral genus-specific protein antigen purified from *L. interrogans* serovar krematos strain Kyoto and canicola strain Hond Utrecht IV, its molecular weight was estimated to be approximately 62 kd.

Thus, it can be concluded that, with rabbit antisera, two specific antigens at Mw of 52 and 46 kd were recognized on non-pathogenic *L. biflexa* serovar patoc whereas three specific pathogenic leptospiral antigens at Mw of 45, 41 and 30 kd were found on all *interrogans*. The common antigens of all leptospires studied were of 60 kd with a doublet 33-34 kd or 32-33 kd depending on the species. Nonspecific reaction was observed on the 48 kd and 89 kd of bataviae antigen utilizing pooled normal rabbit antisera and pooled normal human antisera respectively.

In the human system, immunoblotting analysis using 3 patient's antisera against pathogenic leptospires revealed a 60 kd common antigen and numerous cross-reactive bands on both pathogenic and non-pathogenic leptospiral sonicates. However, our results showed that the two bataviae antisera derived from rabbit and human gave weak reaction on the 60 kd antigen (Figure 5 and Figure 9). It is conceivable that this antigen is a poor immunogen of serovar bataviae. One serovar-specific antigenic band of *L. interrogans* serovar bataviae was observed on Mw of 66 kd (Figure 9). Many specific antigenic bands (Mw of 80, 73, 68, 43 and 30 kd) on pathogenic sonicates were also shown. Only one

identical band at Mw of 30 kd was found in all *interrogans* tested against all homologous and heterologous human anti-leptospiral antisera. This is comparable with the recent finding of Chapman *et al*. (27) who reported a major 28 kd sub-unit of serovar hardjo lipopolysaccharide reacted with all serum samples from patients infected with *L. interrogans* serovar hardjo. The cross-reactivity between *Leptospira* and *Treponema pallidum* was also demonstrated by immunoblotting against a patient's serum infected with *T. pallidum* (positive - TPHA serum). A 60 kd antigen was visible among the six cross-reactive bands. Thus, this 60 kd antigen should not be included as one of the common antigen of leptospira.

In this study, it is concluded that a 30 kd component is a specific pathogenic leptospiral antigen. This antigen may play important roles in the differentiation of pathogenic and non-pathogenic leptospiral strains. Other methods such as 8-azaguanine resistance test (52), growth test at temperature of 13 °c (47) and growth on trypicase soy broth (66) have been recommended in the past. Specific pathogenic leptospiral antigen may be useful in the characterization of *Leptospira*, taxonomy, preparation of specific antibody or monoclonal antibody and vaccine production. Moreover, immunoblotting technique may be applicable in providing another method for the identification of pathogenic from nonpathogenic leptospira in clinical isolates and for detection of leptospiral antibody.

Conclusion:

1. Agglutinating antibodies produced form rabbits immunized with viable leptospires show titers ranging from 1:3,000 - 1:5,000 by MAT.

2. SDS-PAGE protein profiles of four serovars of leptospires revealed a complex pattern with molecular weight range of 25 to 90 kd by Coomassie brilliant blue staining.

3. Immunoblotting technique was developed for the differentiation of pathogenic and non-pathogenic leptospiral antigens with specificity and sensitivity.

4. A 60 kd common antigen and a doublet of 33-34 kd or 32-33 kd were found on leptospires by immunoblotting using rabbit anti-leptospiral antisera.

5. Two antigenic bands, Mw of 52 and 46 kd were specifically found on non-pathogenic *L. biflexa* serovar patoc.

6. Three specific pathogenic leptospiral antigens at Mw of 45,41 and 30 kd was demonstrated using rabbit anti-leptospiral antisera.

7. Numerous specific pathogenic bands (Mw of 80, 73, 68, 43 and 30 kd) were observed using human antisera against various leptospires but only one, of 30 kd, was found in all human antisera. This indicated that there is some differences between rabbit and human immune response against leptospiral infection.

8. Immunoblotting analysis may be useful in providing an alternative method for the identification of pathogenic leptospira from clinical isolates and a rapid method for the detection of leptospiral antibody against specific antigen.