CHAPTER I
INTRODUCTION

Leptospira is the causative agent of leptospirosis, a reemerging zoonotic disease of worldwide prevalence. In Thailand, the case incidence has markly increased from less tan 500 cases in 1996 to about 15,000 in 2000 with mortality rates ranged from 2.53 to 5.31% (Department of Disease Control, Ministry of Public Health, Thailand). The spirochetal bacteria are ubiquitous especially in tropical and temperate areas (Bharti *et al*., 2003). The bacteria can infect both humans and a variety of mammals including cattle, pigs, dogs, cats, rodents and wildlife (Vinetz, 2001). Animal infections may lead to either clinical disease or the bacteria get adapted and maintained in the host, especially in immunologically privileged sites such as the kidneys where they are excreted into the urine. Human infection occurs by direct exposure to the infected animal's urine or carcass, or indirectly by contact with contaminated environment. Bacterial transmission to humans occurs mainly through wounded, abraded or macerated skin and mucous membranes like the conjunctiva. Traditionally, *Leptospira* are classified into two species, *i.e*., *Leptospira interrogans* which includes all pathogenic serovars and *Leptospira. biflexa* which are non-pathogenic and free living (Smibert, 1973; Johnson and Faine, 1984 and Levett, 2001). Within the genus, there are more than 250 different *Leptospira* serovars based on different agglutinating antigens on their cell wall. Strains of antigenically related serovars have been assigned to the same serogroups (Levett, 2001). Recently, molecular techniques, such as 16S rRNA analysis (Hookey *et al*., 1993) and multilocus sequence typing, based on sequence alignments of genes coding housekeeping enzymes (Ahmed *et al*., 2006), have been used for re-delineation of *Leptospira* spp. into genomospecies. These are: *L. alexanderi, L. biflexa, L. borgpetersenii, L. fainei, L. inadai, L. interrogans, L. kirschneri, L. meyeri, L. noguchii, L. parva, L. santorasai, L. weilii, L. wolbachii* and a few others yet to be named genomospecies.

Most *Leptospira* infections in humans are subclinical and only retrospectively recognized by the presence of serum antibodies to the bacteria. A portion of infected

individuals develops different degrees of morbidity ranging from mild, flu-like symptoms which are indistinguishable from other febrile illnesses, to acute and severe illness which may lead to rapid fatality (Plank and Dean, 2000; Bharti *et al.*, 2003). Clinical leptospirosis is commonly manifested in two forms, *i.e.*, anicteric and icteric or Weil's disease. Anicteric leptospirosis is typically biphasic: the initial phase called the acute or septicemic phase is characterized by generalized leptospiremia which may start as early as one day or as late as one month post-*Leptospira*-exposure (Feigin and Anderson, 1998). Symptoms of leptospirosis are non-specific and may include high fever, chill, cough, sudden onset of intense headache, severe muscular pain especially in the calf muscles, abdominal pain, conjunctival suffusion, blurred vision, photophobia, and others (Scott and Coleman, 1996; Feigin and Anderson, 1998). The second phase called the immune phase follows the acute phase about a week later and is characterized by the appearance of specific antibodies in the blood and the disappearance of the leptospiremia as the organisms escape the serum antibody into the immunological privilege sites such as the kidneys. The bacteria are excreted with the urine of the recovered patients for several months (Van Crevel *et al.*, 1994; Kelley, 1998). Icteric leptospirosis is characterized by relatively more severe ailments including generalized vasculitis, jaundice, hemorrhage, myocarditis, aseptic meningitis, vascular collapse and/or hepatic and renal failure resulting in a high mortality especially when treatment is delayed and/or inadequate.

Usually ampicillin, amoxicillin, and doxycycline are used for the mild anicteric form of the disease. Patients with severe anicteric and icteric leptospirosis are commonly treated with intravenous penicillin, ampicillin, erythromycin or amoxicillin (Kocen, 1962; McClain *et al.*, 1984) together with other supportive measures, *e.g.,* peritoneal dialysis and artificial respiration. Delayed diagnosis and improper treatment result in high mortality. Antibiotic treatment is beneficial if started early in the course of the illness. Unfortunately, diagnosis of leptospirosis is rarely made rapidly at that time. Jarisch-Herxheimer reactions (JHR) caused by toxic bacterial substances massively released as a result of the antibiotic mediated-bacterial lysis occurs in a fraction of the treated patients which may aggravate the existing severe clinical manifestations (Friedland and Warrel., 1991; Emmanouilides *et al.*, 1994; Vaughan *et al.*, 1994 and Pound and May, 2005).

Antibody therapeutics has been used for treatment of various infectious and non-diseases especially during acute illness. Nevertheless, most antibodies are derieved from non-human sources such as murine or horse immune immunoglobulins.

After the hybridoma technology developed by Kohler and Milstein in 1975. There is an increasing trend of using mouse monoclonal antibodies as an non-drug immunotherapeutic agent. However, repeated administrations of these non-human molecules often lead to undesirable side effects, for example, serum sickness, anaphylaxis, due to anti-globulin response in recipients (Lobugio *et al*., 1989).

The anti-globulin response is mainly stimulated by the constant regions (Fc portion) of the non-human antibodies. However, by antibody engineering technique, we can construct small and simple antibody molecules without the constant region which we call the "single chain variable fragments (scFvs)". The single chain antibody retains specific antigen binding site to particular epitope and confer better tissue penetrations. Unfortunately, these molecules are still murine origin and can provoke human anti-murine antibody eapecially against murine immunoglobulin frameworks (FRs) after repeated administrations to human.

One strategy to alleviate this problem is to graft the mouse complementarity determining regions or CDRs that are indispensible for antigenic specificity onto human immunoglobulin framework. This approach is called "Humanization" of the murine antibody. This molecule is less immunogenic in human use and still retains the specificity as original murine antibody.

Recently, the murine hybridoma clone LPF1 secreting specific monoclonal antibody (MAb) against pathogenic strains of *Leptospira* was established. The epitope of MAb LPF1 was characterized by Sakolveree *et al*. (2007). It was found to be various proteins of *Leptospira* with different functions. In this study, protective efficacy against *Leptospira*-mediated pathophysiology is tested both *in vitro* and *in vivo.* A humanized-murine monoclonal antibody specific to pathogenic *Leptospira* spp. is constructed and tested for its protective activity both *in vitro* and *in vivo.*