CHAPTER I

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by autoantibody production and immune complex formation. The tissue deposition of antibodies and immune complexes could cause inflammation and injury of multiple organs. SLE patients have symptoms that can affect virtually any organ system with diverse clinical manifestations, including glomerulonephritis, dermatitis, thrombosis, vasculitis, seizures and arthritis. Therefore, individuals affected with SLE can have different disease phenotypes. SLE is a worldwide disease with a prevalence of approximately 1 in 2000 US population but it varies among ethnic groups. The disease primarily affects women in their reproductive years (F:M ratio 9:1) and the estimated prevalence as well as the severity is 2-to 4-fold, a higher in non-Caucasian as compared with Caucasian population (Hochberg 1997). Although, etiology of the disease remains unclear, cumulative studies suggest that interaction of multiple genes and environmental factors result in susceptibility to SLE (Arnett, Reveille et al. 1997); (Vyse and Kotzin 1998); (Kotzin B.L. 1998). Especially, twin studies show the concordance rate of SLE in monozygotic twins is approximately 10 times higher than the rate in dizygotic twins (Arnett, Reveille et al. 1997); (Hochberg 1997). This data implicates the strong genetic factors in SLE.

Studies from a murine model of SLE suggest that the hereditary risk for SLE is dependent on complex interactions of many genes (Wandstrat and Wakeland 2001) (Wakeland, Liu et al. 2001). During the past years, the search for predisposing to SLE in human can be broadly divided into two strategies: the genome-wide linkage studies and candidate-gene-association studies. Several genome scans have been carried out by the four major scientific groups (located in California, Oklahoma, Minnesota and Sweden), revealing 13 major cytogenetic locations show significant evidence of linkage to SLE. Among the identified linkage are eight "Significant linkage" or "Major loci" to SLE. These are 1q23, 1q41, 2q37, 4p16, 6p21, 11p13, 12q24 and 16q13. These linkage results are encouraging, considering that confirmation of significant linkage of a locus offers the strongest evidence for the existence of putative susceptibility genes. Further efforts in fine mapping to narrow the linked loci for the eventual identification of susceptibility genes are necessary (positional candidate gene). Another strategy is candidate-gene-association studies. In candidate gene analysis an allele or haplotype, or any DNA polymorphism, is directly assessed and a difference in frequency of an allele is demonstrated between affected patients and appropriate controls. Therefore, genetic association with a candidate gene suggests that the polymorphisms being measured is the actual disease-causing allele, or one located very closely to the responsible

gene. Several candidate genes have been studied and found to be associated with SLE such as, FcγR2A, FcγR3A (1q23), IL10 (1q31-32), CTLA-4 (2q33), PDCD-1 (2q37), HLA-DR2,-DR3, TNFα, TNFβ (6q21), C4 (6q21), MBL (10q11-21),FASL, FAS (10q24) and Bcl-2 (18q21) (Nath, Namjou et al. 2004).

One of the significant regions reside on chromosome1. Supporting evidence for linkage in this region was provided by the USC and UCLA group. The interval at 1q41-42 shows evidence for linkage in nearly all of the SLE mapping studies reported to date. (Tsao, Cantor et al. 1997) (Tsao, Cantor et al. 1999). This region has also been of interest due to synteny of the lupus NZM2410 mouse localized to the telomeric region of chromosome 1 (*Sle1*) (Morel, Rudofsky et al. 1994). Phenotypic analysis revealed that this locus contributes a unique component phenotype to disease pathogenesis. *Sle1* mediates the loss of tolerance to nuclear antigens with a high specificity for the H2A/H2B/DNA subnucleosome (Morel and Wakeland 1998). Interestingly, the coexpression of *Sle1*, *Sle2*, and *Sle3* as a B6-triple congenic results in severe systemic autoimmunity and fully penetrant, fatal glomerulonephritis (Morel, Croker et al. 2000). The analysis additionally demonstrated that *Sle1*, which breaks tolerance to chromatin antigens, was essential for the development of fatal lupus nephritis in the B6-congenic model system since only combinations of intervals that included *Sle1* developed severe lupus nephritis. A fine mapping analysis of the *Sle1d* congenic interval localize into regions syntenic with 1q41 (Wakeland, Morel et al. 1997; Wandstrat and Wakeland 2001). Candidate genes within this region in human include poly-ADP-ribosyl transferase (PARP) gene, HLXI gene and TGF-ß2 gene (Tsao, Cantor et al. 1999).

The loss of immune tolerance to self-components is the basis of SLE etiology. In addition, SLE is a disorder of generalized autoimmunity characterized by B cell hyperactivity with numerous autoantibodies againt nuclear, cytoplasmic and cell surface Ags. SLE is also a T cell-dependent disease with many examples of T cell dysfunction, that maintain self tolerance. Therefore, the down-regulate B cell function have broken down (Horwitz, Gray et al. 1997). Since spontaneous remissions are frequent in human SLE, dysfunctional regulatory cells may contribute to existence as well as the onset of SLE (Gladman, 1996). Many genes encoding proteins with regulatory or adaptive functions in the immune system have been considered as candidates. One in many genes that interesting is transforming growth factors-beta (TGF-ß2) gene. TGF-ß in SLE have important role especially in regulatory of immune system. TGF-ß *is* an immunomodulater and immuno suppressor (anti-inflammatory cytokine), particularly through their action on macrophages. TGF-ß is an important costimulatory factor in the generation of $CD8^+$ T cells that down-regulate B cell function. TGF-ß is also an immunosuppressive cytokine, as it inhibit T and B cell proliferation (Gray, Hirokawa et al. 1994). In summary TGF-ß is a multifunctional family of cytokines important in tissue repair, inflammation, and immunoregulation (Massague, 1990).

Furthermore, in general TGF-ß inhibits the development of cells that come from epithelial or neuroectodermal but accelerate the development and differentiation of cells that come from mesenchyme such as fibroblast osteoblast and smoot muscle cells. TGF-ß is the chemotactic factor of fibroblast and stimulates extracellular matrix protein. So it have important role in a process of fibrosis (Ohtsuka, Gray et al. 1998). The development of kidney disease in SLE is one predominant factor that control severity in kidney. Occuring of lupus nephritis results from the autoantibodies deposition and immune complex in kidney leading to severe inflammation. Because of parts of TGF-ß synthesis have invole mesangial, endothelial and epithelial cells. The various studies especially in the abnormal from glomerulonephritis indicated that TGF-ß have important roles to carry out renal fibrosis and chronic kidney disease (Grande, 1998).

At least three TGF-ß isoforms exist in mammals; TGF-ß1 (19q13), TGF-ß*2* (1q41) and TGF-ß3 (14q24) (Derynck, Jarrett et al. 1985); (de Martin, Haendler et al. 1987); (ten Dijke, Hansen et al. 1988) but TGF-ß1 is the principal type produced cells of the immune system. Many studies analyzed the association of TGF-ß1 gene polymorphism in various positions in Caucasian and non-Caucasian. However, no association were found with SLE. Less studies about SNP of TGF-ß*2* with SLE. Most reports only study in some positions that not really point to important about SNP with SLE and no reports about SNPs of TGF-ß*2* including function of SNPs with SLE in non-Caucasian. We are interested in the role of TGF-ß*2* gene polymorphisms and genetics susceptibility of SLE in Thai population due to both role in SLE pathogenesis and its gene position which lie in the position (significant linkage 1q41). We accordingly chose TGF-ß*2* as the candidate gene in this study.

The aim of this study was to investigate the polymorphism of TGF-ß*2* gene in patients with SLE compared with control group and determine the association with SLE in Thai population. We are interested in the polymorphism of TGF-ß*2* gene that might influence disease susceptibility and severity, and act as marker for the disease.

We select TGF-ß*2* SNPs in Thai population by searching from ThaiSNP database. We chose 4 SNPs from 7 SNPs in order to represent the SNP within all exons and nearly intron region of TGF-ß*2 gene* by SNP haplotype tagging from DNA pools of two individuals, Pools2 package and then consisder the suitable SNP distribution and frequencies (greater than 5%). The genotyping method for TGF-ß*2* gene polymorphism were done by 5' – end labeling primer (kinase reaction) and PCR-restriction fragment length polymorphism (PCR-RFLP), respectively. Then genotypes, allele and haplotype frequencies were compared between SLE patient and control subjects.

We hypothesized that the specific polymorphism of TGF-ß*2* gene that determine risk for development and severity of SLE in Thai population will be found. This study help contribute to the identification of SLE susceptible gene and might lead to development of new prognostic markers based on these genotypes, in order to

the better understanding of mechanism of SLE and development of new treatment and prevention. In addition, it will provide the frequency of TGF-ß*2* gene polymorphism in Thai population which it basic knowledge for study these markers in other diseases in the future.