# CHAPTER 1

# INTRODUCTION

Systemic lupus erythematosus (SLE) is a non-organ specific autoimmune disease that is characterized by widespread inflammation affecting virtually any organ and/or system in the body, and the production of various autoantibodies, in particular, antinuclear autoantibodies (ANA). The ANA are autoantibodies directed against chromatin and its individual components, double-stranded DNA (dsDNA) and histones, and some ribonucleoproteins. The serological hallmark of SLE is the presence of antibodies against double-stranded DNA. It is generally assumed that these anti-dsDNA antibodies participate in the development of lesion in this disease. The primary event inducing the formation of anti-dsDNA antibodies has always been obscured, since it has been very difficult to demonstrate the presence of free DNA in serum of SLE patients (1). Furthermore, native DNA is usually considered non-immunogenic(2, 3). Mammalian DNA was proved to be not immunogenic, immunization with mammalian DNA did not induce pathogenic anti-dsDNA antibodies(4).

Evidence accumulated in recent years suggests that the nucleosome, the fundamental unit of chromatin and normal product of cell apoptosis, plays a key role in murine and human lupus, as a major immunogen in the induction of pathogenic ANA (5, 6)and as a target antigen for autoantibody-mediated tissue lesions, in particular in kidney(7, 8). In the cell nucleus, 2 meters of DNA are densely packed as chromatin, a polymer structure of nucleosomes interlinked with protein-free DNA(9). Each nucleosome consists of pairs of the histone peptides H2A, H2B, H3 and H4, forming the histone-octamer. Around this octamer 146-160 bp of DNA are wrapped in the two superhelical turns and a molecule of histone H1 is located at the point where DNA enters and exits the nucleosome.

In the recent years, it has become evident that at least in certain murine models of SLE (*lpr* and *gld* mice), and perhaps also in SLE patients as well, the process of apoptosis is aberrant. When a cell becomes apoptotic, the linker DNA is cleaved between the nucleosomes, and nucleosomes combined with other intracellular components will appear at the surface of the cell. Normally, these apoptoitc cells are phagocytosed rapidly, but if this does not occur nucleosomes may be released. Additionally, it has been shown that during apoptosis, a series of posttranslational protein modifications, including proteolysis, phosphorylation, oxidation etc., may create modified autoantigens that might contribute to the bypass of tolerance that is required for autoantibody formation(10). On the other hand, apoptosis of maturing T cells is involved in establishing and maintaining tolerance, and therefore a disturbed apoptosis may cause a breakdown of tolerance.

Identification of the nucleosome as a major lupus-specific immunogen for pathogenic autoantibody-inducing Th cells is a major step toward understanding the etiology of SLE. Nucleosome-specific CD4+ T cells are detected long before lupus mice produce pathogenic autoantibodies, suggesting that these cells plays a role in triggering the disease (5).

Remarkably, a single lupus nucleosome-specific Th clone can provide help to a dsDNA-, histone-, or nucleosome-specific B cell, presumably through binding by each of these B cells to its respective epitope on the entire nucleosome, followed by processing and presentation of the relevant nucleosome epitope to the Th clone, resulting in intermolecular help and cognate interaction between B cells and nucleosome-specific T cells, a concept known as antigen spreading(5).

The central role of nucleosomes for induction of the autoimmune response in SLE is also underlined by the formation of nucleosome-specific antibodies, which react with nucleosomes but not with its constituents DNA and histones. This reactivity was first documented for monoclonal antinucleosome antibodies (mAbs) generated from lupus mice and patients(11), and was also found in a high proportion (>80%) in the sera of lupus mice and patients, before the development of anti-dsDNA and antihistone antibodies(9).

Renal disorders are a cardinal manifestation of SLE, and affect the prognosis and mortality. Extracellular deposition of antibody and complement fixation are clearly sufficient to produce glomerulonephritis(12). Diminished serum concentrations of C3 and C4 are observed primarily in active SLE and the serum concentrations of the complement factors reflect the activity of the disease(13). Moreover, there is strong evidence that antinucleosome antibodies play a role in nephritogenic process; they are present in the kidney elutes of lupus mice with proteinuria(14).

Recent evidence obtained in SLE patients found that the nucleosome is emerging as the most reactive substrate among the nuclear antigens in SLE, 55-80% of lupus patients being positive for antinucleosome antibodies(15, 16). It was shown to be highly specific for the disease(15, 16) and also be specific to the anti-dsDNA-negative SLE (17). Indeed, this antibody population is potentially nephritogenic, its titer correlates with the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score, a validated index of SLE activity(18-20) and these antibodies were shown to be more highly associated with nephritis than were antibodies to DNA (21).

In a study of 120 SLE patients with active and inactive disease(22), they found that antinucleosomes of IgG3 were present at high levels in patients with active SLE and were virtually absent in those with inactive SLE and their levels showed a positive correlation with SLE disease activity and found to be closely associated with active nephritis. In addition, SLE, Scleroderma and mixed connective tissue disease were the only 3 connective tissue diseases (CTD) in which antinucleosome antibodies were detected (22). This suggests that antinucleosome antibodies could be a new marker that may help in differential diagnosis of CTD. However, at present, nucleosome-specific antibodies are not tested routinely in lupus patients.

In the present study we measured the prevalence of specific antinucleosome antibodies in SLE patients and healthy individuals, the correlation of these antibodies to the anti-dsDNA, C3, C4 components of complement, and to disease activity index (SLEDAI) were evaluated. In this regard, the indirect enzyme-linked immunosorbent assay (ELISA) was developed for the detection of antinucleosome and anti-dsDNA antibodies in SLE patients.