CHAPTER 7

CONCLUSION

Sixty-five sera from SLE patients and 115 from healthy controls were recruited to determine the frequency of antinucleosome antibody by using ELISA. Determination of anti-dsDNA antibody by ELISA was detected in the same populations. To detect the quality of nucleosomes as antigen in ELISA system, it revealed that nucleosomes prepared from chicken erythrocyte nuclei consisted of nucleosomal DNA banding at 154 base pairs detected by 1.5% gel electrophoresis and SDS-PAGE analysis demonstrated the nucleosomes had the core histone bands at molecular masses of 16.5k (H3), 15k (H2A), 13.5k (H2B), and 12k (H4), suggesting that this preparation yielded "intact" and highly purified mononucleosome core particles. In this study, the optimal concentration of nucleosomes and dsDNA is 5 μg/ml. For peroxidase-conjugated antihuman IgG, a 1:4000 dilution produces the good results and is chosen in antinucleosome and anti-dsDNA ELISA. Intra- and interassay coefficients of variation by antinucleosome and anti-dsDNA ELISA are belonged to the acceptable range.

For the prevalence of antinucleosome and anti-dsDNA antibodies in 65 SLE patients, the results revealed that 34 (52.3%) in 65 SLE patients had antinucleosome antibodies. While 24 (36.9 %) of 65 patients were found to be positive by the anti-dsDNA ELISA suggesting that the frequency of antinucleosome antibody in SLE patients is higher than in anti-dsDNA antibody in lupus patients ($P = 0.052$).

In correlation study, we found that antinucleosome as well as anti-dsDNA reactivities in SLE patients detected by indirect ELISA were significantly correlated with disease activity by SLEDAI as anti-dsDNA reactivity. Moreover, to compare these two antibodies by ELISA, antinucleosome antibodies were found to be significantly correlated with antibody to dsDNA. According to association with C3

and C4 levels, antinucleosome antibody activity inversely correlated with complement C3 levels, but no correlation was found in association with C4 levels by nephelometry.

The frequency of antinucleosome antibodies in patients with active and inactive SLE were also assessed, in the 45 active SLE, 29 patient sera (64.4%) were positive to nucleosome. Further analysis in SLE patients who had inactive SLE, it revealed that 5 of 20 (25%) patient sera showed antinucleosome antibody positivity. The prevalence of anti-dsDNA antibody in active SLE patients was 46.7% (21 of 45) in this group. In the inactive group, it presented that 3 of 20 (15%) inactive SLE patients were revealed positivity to dsDNA. In conclusion, the positivity of antinucleosome antibodies in this study was revealed higher frequency in active SLE patients than those to dsDNA in the similar group indicating antinucleosome may be a better marker in active SLE than anti-dsDNA antibody.

In the present study we found that 16 of 34 sera (47.1%) were shown to react with nucleosomes detected by ELISA without positivity to anti-dsDNA antibodies measured by the similar assay. Our data suggest that the antinucleosome antibody may be a useful marker for diagnosis in anti-dsDNA negative SLE. Further analysis in active versus inactive SLE patients was determined. Interestingly, in antinucleosome positive group without anti-dsDNA antibodies, it revealed that antinucleosome antibody was shown in 13 of 16 (81.3%) in active SLE patients. We conclude that among 4 SLE disease markers studied in this report including antinucleosome, anti-dsDNA antibody, C3 and C4, we found that antinucleosome antibody was the most sensitive marker for SLE patients and the use of antinucleosome antibody as a marker of disease activity may deserve for diagnosis in SLE patients.