ABSTRACT

The presence of spontaneously occurring autoantibodies to nuclear antigens is a hallmark of diseases collectively known as autoimmune diseases and include systemic lupus erythematosus, progressive systemic sclerosis, rheumatoid arthritis and Sjogren's syndrome. The antigens recognized in these rheumatic diseases include single-stranded DNA, single-stranded RNA, double-stranded DNA, double-stranded RNA, ribonucleoprotein, nucleosomes, histones and nonhistone proteins. Antibodies to DNA are of prime importance in both human and animal diseases. The laboratory detection of circulating anti-DNA antibodies is of great clinical significance not only for the diagnosis of rheumatic diseases but also for the management of patients afflicted with them. A number of techniques have been adopted for the measurement of anti-DNA antibodies but their usefulness is limited by one or more factors.

The incidence of anti-DNA antibodies in various rheumatic diseases was studied by immunoprecipitation and hemagglutination techniques and the sensitivity of these assays was compared with spectrophotometric and fluorometric techniques reported in this thesis. Seventeen percent and 26% of SLE patients were positive for anti-DNA antibodies by immunodiffusion (ID) and counter-immunoelectrophoresis (CIE) respectively as compared to 35% with passive hemagglutination assay (PHA). Antibodies to DNA were positive in 39% and 43% of SLE patients with spectrophotometric
and fluorometric techniques respectively. Anti-DNA antibodies were detected in 14% of SLE patients with overlapping PSS by PHA, spectrophotometric as well as fluorometric techniques. However, none of such sera was positive by immunoprecipitation techniques. These results show that both spectrophotometric and fluorometric techniques are more sensitive than ID and CIE and almost parallel in sensitivity to PHA.

A microplate enzyme-linked immunosorbent assay (ELISA) is described for the measurement of anti-DNA antibodies. The method employs plastic surface for antigen binding and alkaline phosphatase-linked rabbit anti-human IgG for the detection of immune complexes, using colorigenic (PNP-P) and fluorogenic (4MU-P) enzyme substrates. The sensitivity of the two substrates was compared in ELISA under different experimental conditions. Fifty seven percent of SLE patients were positive for anti-DNA antibodies with PNP-P and 71% with fluorogenic substrate. Microplate ELISA as described in this thesis in conjunction with 4MU-P is simple, sensitive and could be of help in the detection of low avidity anti-DNA antibodies.

The sensitivity of ELISA has been further increased by the use of different solid support to increase the surface area for antigen immobilization and by decreasing nonspecificity and background values. We report the successful utilization of nylon as solid phase for antigen binding for use in ELISA in the detection of anti-DNA antibodies. A number of preliminary experiments
were performed to define the experimental conditions for optimum results. The prevalence of anti-DNA antibodies in rheumatic diseases was compared using fluorogenic and colorigenic enzyme substrates. Fifty two percent of SLE sera were positive for anti-DNA antibodies with PNP-P and 56% by 4MU-P as substrates. Fourteen percent of SLE patients with overlapping PSS and 10% of PSS patients showed a positive reaction with both the substrates. ELISA utilizing nylon as solid support is convenient, specific and suitable for use in clinical laboratories where large number of samples are to be screened for the detection of anti-DNA antibodies. Antigen coated beads can be stored for appreciable period without loss in antigenicity. The procedure requires small quantities of antigen or antibody and is therefore highly economical.

Anti-DNA antibodies in the sera of patients with various rheumatic diseases were classified into three types depending upon their avidity, on the basis of their reaction in ID, CIE, PHA and ELISA utilizing nylon as solid phase. Twenty one percent SLE patients, 14% SLE + PSS and 10% PSS patients showed low avidity anti-DNA antibodies. Twenty three percent of SLE patients and 14% SLE + PSS patients demonstrated moderate avidity whereas thirteen percent of SLE patients had high avidity anti-DNA antibodies.

Rabbits immunized with calf thymus ds- and ssDNA-MBSA complex elicited antibodies against ssDNA as detected by immuno-
precipitation and hemagglutination techniques, indicating their specificity for bases and that the antigenic determinants are not related to the backbone of DNA.

Antibodies to native DNA can not be produced by experimental immunization but occur spontaneously in NZB/NZW mice which are genetically predisposed to a disorder closely resembling human SLE. We report the production of antibodies against DNA-psoralen adduct showing crossreactivity with dsDNA. These antibodies were not detectable by immunoprecipitation and hemagglutination techniques. ELISA utilizing nylon as solid support was adapted for the detection of these antibodies. The production of low avidity, conformation oriented antibodies in animals immunized with DNA-psoralen adduct was observed. These antibodies were specific for DNA-psoralen adduct and showed strong crossreactivity with dsDNA. Single-stranded DNA and RNA exhibited low reactivity with immunized sera. SLE anti-DNA antibodies isolated by affinity chromatography using dsDNA-Sepharose 4B columns were used to study the specificity of immune sera. The results showed that affinity purified anti-DNA antibodies have an immunological specificity identical to that of the experimentally immunized sera.

The results presented here suggest that although it is not possible to immunize animals against dsDNA but when DNA is intercalated with psoralen, this apparently nonimmunogenic molecule becomes immunogenic as a result of change in some portion
of DNA molecule induced by intercalation with psoralen. The low reactivity of immunized sera with ssDNA/RNA could probably be due to nonavailability of the antigenic bases to elicit antibody response. It is proposed that similar structural changes might occur in DNA by causative agent(s) making it highly antigenic and thus contributing to the pathogenesis of SLE.