I. INTRODUCTION

The need for self and nonself discrimination was appreciated long ago and the concept of autoimmunity was considered early in the development of immunology. Autoimmunity can be considered as a two-stage process in which a breakdown of immunological regulation activates immunologic effector mechanisms which results in autoantibody formation and tissue destruction.

Immune reactions are mediated by a number of different effector cells. These include B lymphocytes and their plasma cell progeny, T lymphocytes which are active in cellular immunity and delayed hypersensitivity, monocytes and related tissue macrophages. These effector cells are regulated by an intricate network of controlling mechanisms. Autoimmunity may arise not as a primary effector cell abnormality but rather as the consequence of disordered immunologic control. The mechanism of autoimmunity is unknown. It is frequently associated with genetic, immunologic and viral factors that interact with immune system and influence regulation (Talal, 1976, 1978, 1981).

The first attempt to explain how self unresponsiveness might be acquired was put forward by Burnet (1962) as a corollary to his clonal selection hypothesis. This hypothesis postulated that each clone of lymphocytes gives rise to cells making antibody
of only one specificity. He also suggested that antibody diversity is generated by rapid somatic mutation especially in embryonic development. Since some randomly generated clones would necessarily be capable of reacting with self antigens, he surmised that these would be eliminated if they come into contact with the corresponding autoantigen during the critical period of development. Thus all self reactive lymphocytes would be deleted and the rest retained. The above hypothesis for self tolerance is no longer tenable in its simplest form because normal animals show some evidence of autoimmunity including autoantibodies, autoantigen binding B cells and autoantibody secreting plasma cells. These are found even when the autoreactive lymphocytes are known to have been exposed to the corresponding autoantigen from early life (Elson et al., 1979).

Nossal and Pike (1975) revived a hypothesis which proposes that at some stage of their differentiation from stem cells to mature antibody forming precursor cells, B lymphocytes go through a phase during which contact with antigen induces only tolerance and not immunity. This theory is different from Burnet's (1962) in focussing attention on the stage of differentiation of lymphocytes rather than the animal as a whole. The tolerant state induced in differentiating cells is independent of suppressor cells and appears to result from the failure of mature progenitors. This is probably how most self reactive lymphocytes are eliminated.
If most autoreactive B cells are deleted then how do some emerge unscathed? Some may escape because the corresponding antigens are not expressed until after the B cells complete their differentiation or because the concentration of autoantigen is too low. Perhaps the critical factor governing the fate of immature autoreactive B cells is the avidity of antigen binding by their surface immunoglobulin receptors, the higher the avidity then the more complete the irreversible loss of receptors. Support for this contention comes from observations that tolerance in immature B cells is more easily induced with multivalent than with paucivalent hapten-protein conjugates (Metcalf and Klinman, 1976; Elson, 1977) and in high avidity clones than low avidity clones (Nossal et al., 1979).

Of various autoimmune diseases, systemic lupus erythematosus (SLE) is considered to be the prototype. A large varieties of antibodies against nucleic acids, nucleoproteins, cell surface antigens and phospholipids have been studied in this disease. There has been no explanation for the extensive diversity in the serological abnormalities of the disease. Lupus autoantibodies are not as diverse as they seem and are restricted in both their ligand-binding properties and their idiotypic diversity. These findings imply that the process of polyclonal B cell activation, the presumed final common pathway of antibody production in SLE must involve a restricted population of lymphocytes (Schwartz, 1983).
Studies of monoclonal representatives of the major class of lupus autoantibodies - anti-DNA antibodies have disclosed that a single autoantibody can bind to multiple nucleic acid antigens of widely different base composition (Andrzejewski et al., 1981; Koike et al., 1982; Pisetsky and Caster, 1982). Another means of ascertaining the nature and limits of the polyclonally activated population of B cells in SLE is by analysis of the idiotypes of lupus autoantibodies. Several such investigations have been carried out. These investigations strongly suggest that anti-DNA idiotypes originate from a restricted family of germ line genes. Moreover, the study reveals that the population of lymphocytes that can produce anti-DNA antibodies occur not only in lupus prone mice but also in normal mice. The spontaneous production of such antibodies, therefore, must involve the loss of regulatory mechanism that operates either at the level of immunological process or on the expression of immunoglobulin genes (Schwartz, 1983).

The presence of spontaneously occurring autoantibodies to many nuclear antigens occur in disease conditions collectively known as autoimmune diseases which include systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), progressive systemic sclerosis (PSS), Sjogren's syndrome (SS), dermatomyositis (DM), and mixed connective tissue disease (MCTD). Nuclear macromolecules recognized as antigens in these autoimmune diseases include double-stranded deoxyribonucleic acid (dsDNA), single-stranded deoxyribonucleic acid (ssDNA), double-stranded ribonucleic acid
(dsRNA), single-stranded ribonucleic acid (ssRNA), ribonucleo-
proteins (RNP), deoxyribonucleoproteins (DNP), nucleosomes, histone
and nonhistone proteins (Tan and Kunkel, 1966; Arana and Seligmann,
1967; Matioli and Reichlin, 1971; Stollar, 1971; Tan and Lerner,
1972; Hannestad and Stollar, 1978; Douvas et al., 1979a; Lerner
et al., 1981).

Antibodies against dsRNA and DNA-RNA hybrid are found in
majority of SLE patients (Schur and Monroe, 1969; Steinberg et al.,
1969; Hiroshi, 1980). Autoantibodies against ssRNA and double-
stranded synthetic polymer like poly I:C, poly A:U (Eilat et al.,
1978), synthetic ssRNA like poly A, poly C (Koffler et al., 1971;
Pillarisetty et al., 1975), tRNA (Eilat et al., 1976) and
ribosomal RNA (Lamon and Bennet, 1970) have been well established.
Antibodies against heterogeneous nuclear RNA have also been
demonstrated (Ali and Tan, 1979). Autoantibodies against RNA are
also present in the New Zealand mouse, NZB/NZW F₁ hybrid (Eilat
et al., 1977). RNA antibodies show marked specificity for RNA
and do not show any crossreactivity with native DNA. However
crossreactivity was observed with DNA-RNA hybrids (Stollar, 1970).

Antibodies to histones have been reported in 95 percent
of patients with procainamide induced lupus erythematosus
(Fritzler and Tan, 1978), 30 percent of patients with idiopathic
SLE (Kunkel et al., 1960; Tan et al., 1976) and 35 percent of
patients with rheumatoid arthritis (Aitcheson et al., 1980).
These antibodies have reactivity against H₁, H₂A, H₂B, H₃ and H₄ histone subpopulations (Rekvig and Hannestad, 1979). It has been reported that the majority of drug induced lupus patients had antibodies directed against the H₂A-H₂B histone complex (Tan and Portanova, 1981). A number of studies have been carried out to determine the precise antigenic determinants on deoxyribonucleoprotein. It has been shown that when the DNA moiety of deoxyribonucleoprotein is destroyed by digestion with DNase, the antigen is no longer reactive with antibody. Similarly when the histone moiety of deoxyribonucleoprotein is digested with trypsin, the remaining DNA is also no longer reactive with antibody. Because of these and a number of other observations it was postulated that the binding of antibodies to deoxyribonucleoprotein is dependent on the presence of both proteins and DNA but is independent of the structural integrity of the entire deoxyribonucleoprotein (Holman and Deicher, 1959; Tan, 1967; Tahourdin and Bustin, 1980).

A variety of other autoantibodies against tissue extracts have been described in the sera of patients with SLE and other connective tissue diseases. Recent studies have explored the significance of antibodies to nuclear nonhistone (acidic) antigen Sm (Tan and Kunkel, 1966), RNP (Sharp et al., 1972), Ro or SS-A (Clark et al., 1969), La or SS-B (Alspaugh and Tan, 1975; Alspaugh et al., 1976; Akizuki et al., 1977), RAP (Alspaugh and Tan, 1976), PM-1 (Reichlin and Mattioli, 1976; Wolfe et al., 1977), Scl-70.
(Douvas et al., 1979b) and have demonstrated their diagnostic specificity and association with certain clinical features in these diseases. Microsomal (Deicher et al., 1960), lysosomal (Wiederman and Meischer, 1965), ribosomal (Schur et al., 1967; Lamon and Bennett, 1970) and mitochondrial (Doniach et al., 1966) antibodies have been reported in these diseases. Recently antibodies to neurons (Toh and Mackay, 1981), α-interferon (Panem et al., 1982), acetyl choline receptor (Valesini, 1983), poly ADP-ribose (Morrow et al., 1982) and collagen (Gioud et al., 1982) have been reported in SLE.

Antibodies to DNA is of prime importance in both the human and animal diseases (Koffler et al., 1967; Lambert and Dixon, 1968; Andres et al., 1970; Atkins et al., 1972; Landry and Sams, 1973; Tan, 1976; Swaak et al., 1982). The formation of antibodies to DNA generally occurs in SLE, New Zealand hybrid mice (NZB/NZW), Palmerston North mice, MRL and BXSB mice (Talal and Steinberg, 1974; Walker et al., 1978; Sawada and Talal, 1979; Kolaja and Fast, 1981; Hahn, 1982). Anti-DNA antibodies have been shown to be particularly injurious type of antibodies in terms of production of inflammatory vasculitis in the kidneys of patients with SLE (Tan and Kunkel, 1966). The presence of DNA and antibodies to DNA in circulation was the initial evidence which suggested that these (DNA-anti-DNA) immune complexes might be important in the kidney diseases. Renal involvement is a frequent and serious feature of SLE involving approximately 75
percent of the patients (Tan and Kunkel, 1966; Koffler et al., 1971; Stollar, 1975; Steensgaard and Johansen, 1980; Yamada et al., 1982).

SLE sera contain heterogeneous populations of anti-DNA antibodies (Arana and Seligmann, 1967; Koffler et al., 1971). Although antibodies to native DNA appear to be almost diagnostic of SLE, antibodies to ssDNA are more widespread (Hughes et al., 1971; Koffler et al., 1971). Recently antibodies specific for Z-DNA have been reported in SLE (Lafer et al., 1983). Autoantibodies to DNA occurring in both human and murine SLE sera can be divided into three major groups on the basis of specificity.

1. Anti-dsDNA antibodies reactive with only dsDNA. These antibodies do not react with single-stranded DNA and therefore it has been presumed that the reactive antigenic determinants on DNA should be related to the double helix conformation. This phenomenon has been checked by studies of binding properties of hybridoma anti-DNA antibodies showing striking specificity for dsDNA (Arana and Seligmann, 1967; Koffler et al., 1971; Andrzejewski et al., 1981; Ballard, 1981; Hahn, 1982).

2. Anti-dsDNA antibodies reactive with both ds- and ssDNA. This type of autoantibody is present in 50 to 70% of patients with SLE. Low concentrations of this antibody are also present in other rheumatological diseases. Because the antibody appears to recognize similar antigenic determinants present on double-
stranded and single-stranded DNA, most workers postulated that the antigenic determinants should consist at least in part of the deoxyribose phosphate backbone of DNA. The dual reactivity of these antibodies with both ds- and ssDNA may be due to the fact that most dsDNA strands contain single-stranded nicks and most of the ssDNA strands have helical areas. A unique feature of antibodies of this type is the fact that they occur spontaneously in SLE and other diseases but do not appear to be immunogenic in experimental animals (Notman et al., 1975; Samaha and Irvin, 1975; Stollar and Papalian, 1980).

(3) Anti-DNA antibodies reactive with only ssDNA. These antibodies recognize purine and pyrimidine bases exposed in ssDNA as antigenic determinants. These autoantibodies are found in patients with rheumatological diseases as well as nonrheumatological diseases such as chronic infectious processes (Stollar et al., 1962; Levine and Stollar, 1968; Alarcon-Segovia et al., 1970; Lacour et al., 1973, Stollar, 1973). Antibodies to ssDNA cross react extensively with RNA (Levine and Stollar, 1968).

Antibodies in autoimmune diseases appear to belong primarily to the IgG class of immunoglobulins. Antibodies to DNA and antinuclear factors belong to both the IgM and IgG immunoglobulin classes. It is clear from murine and human studies that renal disease and disease activity in general are more closely related to high circulating levels of IgG anti-DNA antibodies.
than to IgM antibodies. In mice it is at the time when production of IgM anti-DNA switches to IgG that proteinuria and declining renal function begin. There has been recent report claiming that antibodies to native DNA in patients with SLE are primarily of IgG4 and IgG3 subclasses (Tan and Vaughan, 1979). These subclasses of IgG are known to be associated with high complement fixing activity. IgG antibodies to DNA which are capable of fixing complement are more likely to be associated with clinically significant renal lesions than are noncomplement fixing antibodies (Arana and Seligmann, 1967; Rothfield and Stoller, 1967; Schur et al., 1972; Sontheimer and Gilliam, 1978a; Tan and Vaughan, 1979; Hahn, 1982; Weinstein et al., 1983).

Native DNA is a poor immunogen and its immunogenic form has not yet been identified. Chemical modifications of DNA (with suitable carrier) leads to the immunogenicity, and higher levels of specificity are due to such modifications of structures (Stollar, 1973, 1981). Normal animals have a remarkable tolerance to DNA and are only capable of making antibodies to ssDNA when complexed to methylated bovine serum albumin and presented in Freund's adjuvant. With suitable protein carriers, ssDNA and synthetic polynucleotides can induce antibody formation in normal animals (Plescia et al., 1964, Stollar, 1973). It has been shown that DNA irradiated with UV light is a potent immunogen and antibodies formed react only with UV irradiated DNA showing no cross reaction with native or ssDNA (Tan, 1968; Natali and Tan, 1971).
conflicting results (Steward et al., 1975; Leon et al., 1977; Tron and Bach, 1977; Winfield et al., 1977; Griffiths et al., 1978; Aarden et al., 1979). SLE sera contained at least two distinct populations of anti-dsDNA antibodies. One population was of rather high avidity and dissociated slowly in the presence of excess DNA or high salt. The other population was of considerably lower avidity and dissociated more rapidly under these conditions. The results of a double label dissociation kinetics study provided independent evidence supporting this hypothesis (Riley et al., 1980). Using dsDNA fragments of varying sizes (20 to 1200 bp), the heterogeneity of anti-DNA antibodies in SLE sera and the recognition of the secondary structure of antigen for specific binding was established. Competitive assays with mononucleotides, oligonucleotides, homopolymers and RNA-DNA hybrid indicated that two strands of polydeoxyribonucleotide were required for optimal reaction with these SLE serum antibodies (Ethel and Gunnel, 1980; Papalian et al., 1980; Stollar and Papalian, 1980).

It has been reported that guanosine is the best nucleoside for inducing antibody production in BALB/c mice (Stollar and Borel, 1976). Accumulation of guanine-cytosine enriched low molecular weight DNA fragments have been shown in lymphocytes of patients with SLE (Sano et al., 1983). G-C rich DNA has been known to form unusual conformations such as triple-stranded, left-handed (Z-DNA) and bent-DNA (Podder, 1971; Selsing et al., 1979; Wang et al., 1981). If such specific structures take place in the
antigen fragments, it is possible that antibodies are raised against them. Two classes of DNA fragments have been isolated from DNA anti-DNA immune complexes by electrophoresis. The small fragments averaging molecular weight 25,000 with a length of 30 to 40 base pairs (bp) and the large fragments averaging molecular weight 100,000 with a length of 150 bp. The small fragment contains an average of 55% G-C content and the large fragment contains 45% G-C content. Because average G-C content in total human DNA is 38%, it was concluded that the antigen is rich in G-C content (Sano and Morimoto, 1982).

The heterogeneity of DNA antibody populations in human and murine SLE sera makes it difficult to study physicochemical characteristics and antigenic specificity. Therefore, the development of hybridoma technology by various workers represents a major advance and studies of monoclonal antibodies to DNA and other nuclear and cytoplasmic antigens are entering a new era.

Serological methods are playing an increasingly important role in the diagnosis and epidemiological assessment of various rheumatic diseases. With the proper assay system the diagnostic value of antibodies to dsDNA seems to be beyond doubt. Antibodies to DNA are found in high frequency in serum of patients with active SLE but not in the serum of normal person and patients with a variety of other autoimmune diseases such as SS, RA and MCTD (Pincus et al., 1969; Hasselbacher and Leroy, 1974; Rochmis
et al., 1974; Aarden et al., 1975; Lightfoot and Hughes, 1976; Bustin et al., 1982; Swaak et al., 1982).

A number of techniques have been developed for the measurement of antibodies to DNA which include complement fixation (Robbins et al., 1957), double diffusion in agar (Seligman, 1957), latex particle agglutination (Christian et al., 1958), counter-immunoelectrophoresis (Davis and Winfield, 1974), hemagglutination (Inami et al., 1973), precipitation of radiolabelled DNA-anti-DNA immune complexes with ammonium sulfate (Farr, 1958; Wold et al., 1968; Pincus et al., 1969), nitrocellulose membrane technique (Kredich et al., 1973), millipore filter assay (Ginsberg and Keiser, 1973), peroxidase labelled antibody test (Vladutiu, et al., 1979), test utilizing glass fiber filter (Lewis et al., 1973), polyethylene glycol precipitation assay (Riley et al., 1979; Smeenk et al., 1982), solid phase radioimmunoassay (Tan and Epstein, 1973; Smith et al., 1981), immunofluorescence (Sontheimer and Gilliam, 1978b; Somerfield and Wilson, 1980), fluorometric assay (Shepherd et al., 1978) FIAX fluorometric assay (Berne et al., 1982) and enzyme-linked immunosorbent assay (Hillyer and Modeline, 1980; Labrouce et al., 1980; Miller et al., 1981; Kawai et al., 1982; Eaten et al., 1983).

The laboratory detection of circulating DNA antibodies is of great clinical significance not only for the diagnosis of SLE but also for evaluating its prognosis and efficacy of treatment (Pincus et al., 1969; Feltkamp, 1975; Swaak et al., 1982).
Sensitive, rapid and economic procedures are desired for clinical diagnosis and efficient management of these patients. The potential usefulness of most of the techniques presently in use is often limited by one or more factors. These are lack of sensitivity, specificity, complexity, difficulty in quantification, presence of serum inhibitors and length of time necessary to obtain results. There is a great deal of conflicting data concerning the clinical significance of anti-DNA antibody titer. These differences may be attributed in part to variations in the methods employed to detect anti-DNA antibodies. Different laboratories have used different types of DNA as antigen and have used either ammonium sulfate precipitation or cellulose nitrate filtration to separate bound from unbound antigen. It has been shown that both of these variables, antigen and means of detection, can result in a significant difference in the amount of anti-DNA antibody measured in the same serum (Aarden et al., 1975, 1976b).

At present there are three widely accepted assays for the detection of anti-DNA antibodies. They are immunofluorescence, radioimmunoassay and enzyme-linked immunosorbent assay (ELISA). Immunofluorescence assay now widely used, employs the kinetoplast of Crithidia lucililae as a substrate. It is specific for dsDNA antibody by virtue of the fact that the circular DNA of the organism appears to be free of single-stranded DNA and histone contaminations (Sontheimer and Gilliam, 1978b). In this assay
smears of *Crithidia luciliae* are incubated with sera to be tested followed by fluorescein labelled anti-human immunoglobulin serum. Fluorescence of the kinetoplast indicates the presence of antibodies to dsDNA. Sera of SLE patients almost always have antibodies reacting with cell nuclei i.e. antinuclear antibodies (ANA), therefore a nuclear fluorescence commonly occurs in the *Crithidia luciliae* assay. The nuclear fluorescence is often difficult to differentiate from the kinetoplast fluorescence, especially when sera are diluted for titration purposes (Vladutiu et al., 1979). In practice immunofluorescence is not easy to quantify for antibody assays since it depends on subjective visual assessments of fluorescence and the results are usually expressed as the serial dilutions of serum that gives least fluorescence.

The most extensively used technique for the detection of anti-DNA antibodies in terms of sensitivity and diagnostic specificity is the radioimmunoassay (RIA) of Farr (1958). Although it is a highly sensitive technique, nevertheless expensive and involves the use of sophisticated radioactive measuring instruments which limit its use in laboratories where all these facilities do not exist. Both advantages and disadvantages are derived from radioactive nature of its label (Schall and Tenoso, 1980). Farr assay has recently come under criticism for alleged inability to detect low avidity anti-DNA antibodies. A small percentage of antibody binding was not detected when this assay was performed under the conditions generally used. Conditions of incubation,
buffer, pH, concentration of reactants and molecular weight of antigen are very important for optimal detection of anti-DNA antibodies by ammonium sulfate assay (Rubbin et al., 1978). The high salt concentration of the ammonium sulfate precipitation dissociates all DNA/anti-DNA complexes of low avidity (Griffiths et al., 1978, Aarden et al., 1979).

The introduction of enzyme immunoassays originally described by Engvall and Perlmann (1971, 1972) and van Weemen and Schuurs (1971) has offered an alternative to the RIA, contributing major improvements in reagent stability, waste handling and safety (Schall and Tenoso, 1980). The range of application of ELISA is potentially as wide as that of RIA and they may also reinforce or replace other serological tests such as immunofluorescence. Automation has been applied effectively, although as with RIA, the definitive system has not been reported (Voller et al., 1976, Schall and Tenoso, 1980). Enzyme immunoassays employ antibodies or antigens conjugated to enzymes in such a way that the immunological and enzymatic activity of each moiety is maintained. These assays give objective results and are extremely sensitive. The evaluation of results can either be visual or be made with a rather simple spectrophotometer of the type found in most laboratories (Voller et al., 1976). ELISA has been described for the measurement of anti-DNA antibodies in SLE by various workers (Miller et al., 1981; Kavai et al., 1982; Eaton et al.,...
The sensitivity of ELISA can be limited by the factors which include the type of solid phase used for antigen immobilization and method used to monitor enzyme activity. In order to achieve the full potential of such assays, a number of modifications have been made. These include, the use of different materials as solid support to increase the surface area for immobilization of antigen, use of fluorogenic enzyme substrate to monitor enzyme activity and modification in the assay procedure and data expression. Such changes have resulted in the increased sensitivity, decreased nonspecificity and background values and ease in performing the techniques (Hendry and Herrmann, 1980; Schall and Tenoso, 1980; Aitkaci et al., 1981; Labrousse et al., 1982; Malvano et al., 1982; Ishaq and Ali, 1983).

Enzyme immunoassay utilizing nylon for the covalent immobilization of antibodies has been reported (Hendry and Herrmann, 1980). Treatment of nylon with HCl for limited time causes exposure of free amino and carboxyl groups. Such an activated nylon is highly reactive, since a number of macromolecules like DNA, RNA and proteins can be noncovalently adsorbed on its surface.

Ethidium bromide (2, 7 diamino-9-phenyl-10-ethyl phenanthridinium bromide), a cationic dye which intercalates strongly and specifically with double helical DNAs and RNAs,
widely used in spectrofluorometric studies because of the striking fluorescence enhancement it displays upon binding (Waring, 1965; LePecq and Paoletti, 1967; Gatti et al., 1975). It is generally agreed that strong fluorescence enhancement accompanies intercalation of the dye into the double helix conformation of the nucleic acid but there is also evidence for additional nonintercalative, less fluorescence enhanced sites which are presumed to involve electrostatic binding (Waring, 1965; LePecq and Paoletti, 1967). One dye molecule is bound for each 2–2.5 base pairs of DNA (Mandal et al., 1980). Ethidium bromide (EB) has been used to probe dsRNA sequences within hnRNA from HeLa cells (Paoletti et al., 1980), tRNA structure (Urbanke et al., 1973), ssRNA (Gray and Saunders, 1971; Feunteun et al., 1975), circular DNA (Hudson et al., 1969), chromatin structure (Ide and Baserga, 1976), ribosomal RNA (Lawrence and Daune, 1976), synthetic DNA (Aktipis and Marts, 1974), tRNA protein interactions (Rigler et al., 1971), determination of molecular weight of DNA (Weissman et al., 1976) and in assaying conformational changes of DNA in DNP (Angerer and Moudrianakis, 1972).

When EB intercalates into a double helix, both its solvent environment and equilibrium conformation are modified, and either one or both of these changes might be involved in the enhancement mechanism. It has been suggested that the fluorescence enhancement is due to the immersion of EB in the hydrophobic region of the nucleic acid where it is protected against the aqueous solvent
(LePecq and Paoletti, 1967). Burns (1969) attributed the enhancement to a change in the conformation of EB.

Shepherd et al., (1978) reported EB assay for the quantitation of anti-dsDNA antibodies using IgG fractions of SLE sera. Their technique was based on the inhibition of fluorescence by the dye after displacement from DNA as a consequence of immunoglobulin binding. Since EB intercalates only with double-stranded nucleic acids, only those antibodies which bind dsDNA should prevent intercalation of EB. The amount of dye displaced is proportional to the amount of IgG bound, making direct linear quantitation of anti-dsDNA antibodies.

The presence of antinuclear antibodies in the sera of patients treated with furocoumarins plus 365 nm light (PUV-A photochemotherapy) indicates a disturbed immunological function as exemplified in various rheumatic diseases (Kubba et al., 1981). The finding that PUV-A therapy is effective treatment for several diseases (mycosis fungoides, alopecia areata, vitiligo) has prompted investigators to study the influence of PUV-A on host immune functions (Grekin and Epstein, 1981). It is suggested that furocoumarins plus UVA irradiation does indeed have a profound effect on the immunological system. Lymphocytes taken from patients treated with PUV-A had decreased phytohemaglutination induced DNA synthesis as compared to nontreated control (Friedman and Rogers, 1980). PUV-A may suppress immunological
surveillance in the skin (Bridges and Strauss, 1980). In vitro, the response of T lymphocytes to HLA-D antigen is inhibited if either the stimulator or responder cells are pretreated with PUV-A. PUV-A has a lethal effect in vitro on lymphocytes and monocytes, but not on the majority of the epidermal cells, suggesting that the beneficial effect of PUV-A on patients with psoriasis may be due to an effect on immunocompetent cells rather than on the epidermis (Morhenn et al., 1980).

The furocoumarins, belong to a group of heterocyclic compounds, formed by condensation of coumarin and furan ring. Many natural and synthetic furocoumarins have been synthesised. Of these furocoumarins, psoralen, 8-methoxypsoralen and 4,5,8-trimethylpsoralen are used clinically in the treatment of vitiligo (Fowlks, 1959; Kaufman, 1960; Scott et al., 1976).

It is well known that two types of interactions may occur between a furocoumarin and DNA: (i) formation of a molecular complex, in the dark, consisting of an intercalation of a furocoumarin molecule, in its ground state, between 2 base pairs of DNA, involving very weak forces such as Vander Waal's forces, hydrogen bonding and hydrophilic forces; (ii) photochemical reaction, following irradiation with UVA light, leading to a covalent combination of furocoumarin molecule to pyrimidine bases of DNA (Dall'Acqua et al., 1979; Hearst, 1981; Sherman and Grossweiner, 1981). Only pyrimidine bases thymine, cytosine or
uracil, their nucleosides and nucleotides react with furocoumarins (Musajo et al., 1965; Musago et al., 1966; Krauch et al., 1967; Pathak and Kramer, 1969). No evidence has been obtained from chromatographic or labelled radioactive tracer studies that furocoumarin react with purine bases, adenine or guanine and their nucleosides (Musajo et al., 1965).

It has been suggested that the drugs with the ability to form complexes with DNA and DNP are most likely to induce the ANA formation (Grabar, 1974). Antibodies against histones, ssDNA, nuclear ribonucleoprotein are detectable in patients treated with procainamide and hydralazine (Stollar, 1981). So far no report of the occurrence of anti-dsDNA antibodies in such patients have appeared.

Antinuclear antibodies have been reported in patients undergoing PUVA therapy. However, antibodies to dsDNA have not been detected, although it is known that psoralen forms a covalent photoadduct with DNA in the presence of UVA light and makes it immunogenic (Zarebska et al., 1978). In such studies Farr assay was employed for the detection of antibodies which is under criticism for its inability to detect anti-DNA antibodies of low avidity.

The present work describes the detection and quantitation of anti-DNA antibodies in the sera of patients with various rheumatic diseases and rabbits immunized with ds- and ssDNA by
immunoprecipitation and hemagglutination techniques. The sensitivity of these techniques was compared with spectrophotometric and fluorometric method which were developed in the laboratory for anti-DNA antibodies. An enzyme-linked immunosorbent assay (ELISA) is described for the assay of anti-DNA antibodies employing plastic surface for antigen binding. Experiments were performed to compare the sensitivity of colorigenic and fluorogenic enzyme substrates under different experimental conditions. We report the successful utilization of nylon as solid support for antigen immobilization for use in ELISA in the detection of anti-DNA antibodies. The production of low avidity antibodies against DNA-psoralen adduct showing crossreactivity with dsDNA have also been described. The antigenic specificity of affinity purified anti-DNA antibodies from positive SLE serum was compared with the antibodies elicited by immunization of animals with DNA-psoralen adduct.