Reactive oxygen species (ROS) have been known to cause damage to a variety of biological substances especially DNA. The damage to DNA is known to be mutagenic and probably represents a major natural hazard for the genomic stability of living cells. The most reactive of the ROS is the hydroxyl radical (•OH) which can attack virtually any target molecule it encounters. These are formed in biological systems via ionizing radiations or through the Fenton reaction. The hydroxyl radical may attack DNA at either the sugar or the base, ultimately causing DNA single strand breaks and formation of altered bases.

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease of unknown etiology characterized by the presence of autoantibodies in circulation that bind to numerous self antigens such as DNA, RNA, ribonucleoproteins and histones. Although antibodies that react with B-conformation are found in sera of patients with SLE, B-DNA per se is a poor immunogen. On the other hand various other conformations of DNA such as right handed A-form and left handed Z-form are potentially immunogenic. Also, chemically modified DNA has been found to result in the production of antibodies. Thus it could be suggested that a conformational variant of B-form is essential for the generation of anti-DNA antibodies.

In the present study purified native calf thymus DNA was modified with hydroxyl radical generated by UV irradiation in presence of hydrogen peroxide. UV spectroscopy of the ROS-modified DNA revealed a significant decrease in the absorbance at 260 nm as compared to its corresponding unmodified control. Thermal denaturation study showed a net decrease of 13.5°C in the Tm value for ROS-DNA indicating a partial destruction of its secondary structure. The single strand breaks induced in DNA as a result of modification by ROS were
detected by alkaline sucrose gradient centrifugation and nuclease S1 sensitivity assay. In addition to the alterations within sugar phosphate backbone, hydroxyl radical was also found to induce DNA base modifications. Ion exchange chromatography on DEAE-Sephadex A-25 column showed the modification of thymine to the greatest extent followed by guanine, cytosine and adenine.

In addition to the modification of DNA by ROS, individual bases adenine, thymine and the synthetic homopolymers poly(dA) and poly(dT) were similarly modified with ROS. UV absorption profile exhibited drastic changes in ROS-modified adenine and thymine, while homopolymers poly(dA) and poly(dT) modified with ROS showed substantial decrease in UV absorbance at 260 nm.

Monoclonal antibodies raised against 400 bp ROS-DNA were used for further studies. A high binding monoclonal (P3C11D6) antibody was chosen. The monoclonal antibody showed a high inhibition of 84% when ROS-DNA was used as inhibitor and a lower inhibition of 60% with 200 bp ROS-DNA. The antibody also showed cross-reactivity with nDNA, showing an inhibition of 65%. The specificity was further ascertained using a variety of inhibitors. Among the bases, ROS-thymine was found to be the potent inhibitor of anti-ROS-DNA antibody binding to the antigen, followed by ROS-guanine. Their unmodified forms were non-inhibitory. Similarly, the other DNA bases i.e. cytosine and adenine were ineffective inhibitors in both their native and modified forms. The homopolymer poly(dT) modified with ROS as well as ROS-thymidine exhibited substantial inhibition although their native forms were non-inhibitory. Some degree of cross reactivity was also observed with some synthetic polynucleotides having A-, B- and allied conformations although to a lesser degree than to the ROS modified epitopes. Thus it appears that the monoclonal antibodies are
specific towards ROS-epitopes and some amount of cross reactivity seen with B-DNA and synthetic polynucleotides could be due to certain shared/common antigenic determinants.

The participation of lysine residues in the immunochemical reactivity of anti-ROS-DNA antibodies was investigated by modifying the free amino groups of lysine residues with acetic anhydride. It was found that the modification of lysine residues paralleled loss in antibody activity, thus suggesting the role of lysine residues in the antigen binding site of the immunoglobulin molecule.

In the present study, SLE sera from patients having high titre anti-DNA autoantibodies were studied for their recognition of ROS-DNA. The binding specificity of SLE autoantibodies was analysed by inhibition ELISA and gel retardation assay. The ROS-DNA showed an appreciable binding with all of the SLE sera tested with enhanced inhibitions in most of the cases compared to the native conformer. In addition, it was observed that ROS-thymine was an effective inhibitor of anti-DNA autoantibody binding to the antigen, as was ROS-poly(dT) but to a lesser extent. In contrast, their unmodified forms were not inhibitory and so was adenine as well as ROS-adenine. The homopolymer poly(dA) and ROS-poly(dA) also failed to show any binding with the SLE autoantibodies. The binding patterns of these autoantibodies obtained from different patients indicate their recognition of altered/modified conformation. The results were further confirmed by dot blot assay and gel retardation assay.

Monoclonal anti-ROS-DNA antibody was used as a probe to assess oxidative DNA damage in SLE patients. DNA isolated from the lymphocytes of SLE patients was used as an inhibitor in competition ELISA. It was found that five out of the eight patients tested showed inhibition of monoclonal antibody
binding to ROS-DNA, thus suggesting the presence of oxidative lesions in these patients which are being recognized by the monoclonal antibodies.

ROS-DNA was found to be a potent immunizing stimulus, inducing high titre anti-ROS-DNA antibodies in rats as evaluated by direct binding ELISA. Kidney sections of the immunized rats were tested for immune complex deposition by immunofluorescence. There was found to be a concentration of immune complexes in the glomeruli of the kidneys which was exhibited as apple green fluorescence. The immune complex deposition may cause kidney inflammation and tissue destruction. This could be correlated to acute stage of lupus nephritis wherein deposition of immune complexes between DNA and antibodies to DNA has been considered to elucidate inflammatory reaction.

In conclusion it could be summed that native DNA modified by ROS is rendered immunogenic in rats and immune complex deposition in kidney could be detected. Also monoclonal antibodies were found to be specific towards ROS-epitopes with special affinity for ROS-thymine and ROS-poly(dT). A similar observation was made when anti-DNA autoantibodies from SLE sera were probed for their binding with other inhibitors besides native DNA. Thereby suggesting that probably thymine in DNA upon modification with hydroxyl radical undergoes conformational changes rendering DNA immunogenic leading to the production of circulating antibodies cross reacting with native DNA in SLE. Monoclonal antibody was also found to be a good probe for assessing oxidative damage to the human genome in systemic lupus erythematosus.