DISCUSSION

The double helical DNA has considerable conformational flexibility. It is not a static molecule but rather a dynamic structure in which different conformations are in equilibrium with each other. The discovery of left-handed Z-DNA has added yet another complexity in the conformation of native DNA. Z-DNA is a strong immunogen, in contrast to B-DNA which is almost nonimmunogenic.

Bromination of double stranded calf thymus DNA in 4M NaCl converts some portions of DNA from right handed conformation to left handed Z- conformation. Synthetic poly(dG-dC).poly(dG-dC) has been shown to attain Z-conformation in 4M NaCl and was stabilized in low ionic environment of a physiological medium by chemical bromination (Moller et al, 1984, Lafer et al, 1981). In case of poly(dG-dC), bromine reacted largely with the C-8 position of guanine and to a lesser extent with the C-5 position of cytosine (Nordheim et al, 1981; Moller et al, 1984). The bromine atom at C-8 sterically prevents guanine from adopting the anti-conformation (Bugg and Thewalt, 1969; Travele and Sobell, 1970). Z-DNA conformation is stabilized when approximately, 35% of the guanine residues in the C-8 position and 17% of the cytosine residues at C5 position are brominated (Moller et al, 1984).

Native DNA brominated in high salt concentration was judged by changes in UV absorption spectrum, Tm value and reactivity with S1 nuclease. As a consequence of bromination in high salt nDNA showed altered absorption in UV region, similar to that observed with brominated poly(dG-dC).poly(dG-dC) (Pohl et al, 1972). The hypochromicity at 260 nm and hyperchromicity at 295 nm with red shift is one of the characteristic feature of Z-DNA (Rich et
et al, 1984; Latha and Brahmchari, 1986). The absorbance ratio for native DNA brominated in high salt was similar to the reported value for the Z-form of poly(dG-dC).poly(dG-dC).

Thermal denaturation data confirms the above results and indicates that Br-DNA is thermodynamically less stable than its corresponding unmodified polymer. The thermal behaviour of native DNA (nDNA) as control displaying Tm value of 88°C is typical of double helical calf thymus nDNA. On the contrary, Tm of brominated DNA (Br-DNA) was found to be 83°C. The substantial decrease in Tm value was due to the fact that helix is destabilized as a result of bromination. The results of time dependent denaturation of native DNA showed a large lag period prior to its denaturation as compared to Br-DNA. This can be ascribed to the denaturation of a 'segment' involving the brominated sites which consequently initiates the rapid denaturation of the Br-DNA. Bromination, disrupts the base pair interaction at the bromination sites thereby generating local denaturation consequently resulting in the destabilization of the helix as compared to native polymer.

The results of nuclease digestion studies indicate a conformational change from B -> Z-form. Nuclease Sl is known to recognize and cleave some structural feature at the junction between its neighbouring right and left handed DNA regions (Singletonet et al, 1982). The increased electrophoretic mobility of Br-DNA as compared to nDNA after nuclease Sl treatment, in agarose gel electrophoresis indicates the generation of some single stranded portions in Br-DNA. The low molecular weight bands indicates that some portions of brominated polymer are digested on the treatment with Sl nuclease.

The separation of modified bases gives an insightful confirmation that the modification incurred as a result of bromination of nDNA. Cytosine and guanine were modified to
the extent of 20.8% and 39.2% respectively. The suggests that guanine is a better substrate than cytosine for bromination. Our results are close to the aforementioned results of brominated poly(dG-dC).poly(dG-dC) in which nearly same amount of guanine and cytosine has been modified.

Immunological experiments were carried out which demonstrated that Br-DNA was highly immunogenic inducing high titer antibodies in goat as analyzed by direct binding ELISA. Similar observations were obtained with brominated poly(dG-dC).poly(dG-dC) which proved to be a potent immunogen inducing about 1.2 mg of antibodies per milliliter of serum (Lafer et al, 1981). To isolate the immunogen specific anti-Br-DNA antibodies from total IgG populations, the sample was passed through immunoaffinity column. Conventional ligand of nucleic acid with CNBr activated Sepharose 4B has been avoided due to a number of inherent limitations. Instead, the antigen was linked noncovalently to Sepharose 4B-polylysine.

Efficient binding of Br-DNA to the matrix was noted and the IgG eluted from the affinity column showed appreciable binding to the immunogen. The formation of immune complex was detected by gel retardation assay. The retarded mobility of Br-DNA and anti-Br-DNA antibodies was the result of formation of immune complex whereas no retardation was seen in case of antigen alone or with preimmune antibody. This indicates that antibodies were generated against Br-DNA.

The modified polymer was highly immunogenic inducing high titer antibodies in goat as analyzed by direct binding ELISA. The specificity of polyclonal, electrophoretically pure anti-Br-DNA IgG was ascertained by competition ELISA using a variety of DNA polymers. Maximum inhibition (96%) was achieved by the immunogen. The remarkable recognition
of anti-Br-DNA antibodies by Br-DNA reveals the highly specific character of the induced antibodies. The presence of conformational specific antibodies in the polyclonal immune sera was evident from the appreciable inhibition in the antibody activity by poly(dG-dm\textsuperscript{5}C).poly(dG-dm\textsuperscript{5}C), which is known to exist in the Z-form in solutions under physiological conditions. Thus, the recognition of this conformer by anti-Br-DNA antibodies is suggestive for the presence of antibodies against Z-like conformation in the immune sera. Moreover, the low binding with single stranded DNA and UV irradiated DNA speculated for the presence of a very small population of antibodies directed against these nucleic acid variant. The binding of ssDNA may be attributed due to the generation of single stranded portions (3-4 base pair) at the B-Z-junctions, thereby giving rise to single stranded determinants which may consequently be responsible for the generation of anti-single stranded populations of antibodies. The non-inhibitory nature of the antibodies with nDNA suggests that the antigenic determinants does not lie on the sugar phosphate backbone of the nucleic acid.

However, forty percent inhibition in the antibody activity by Br-RNA speculates for the possible involvement of the modified bases which may act as a potential epitopes, responsible for the induction of a small population of antibodies directed against them. Poor inhibition was obtained with poly(dA),poly(dT) and poly(dC) again shows the non-reactivity of antibodies with unmodified bases.

Appreciable inhibition of antibody binding to Br-DNA was obtained with poly(dG).poly(dC) and poly(dA).poly(dT). The possibility for the involvement of sugar phosphate backbone in antigenicity has already been ruled out as evident from the above results. Thus the appreciable inhibition in the antibody activity with these polymers might be due to recognition of the new conformation provided
by the polymers, since polypurines in one strand and polypyrimidines in the other strand possess neither A-type nor B-type conformation. They provide a unique conformation which is in between the A and B-type. Thus, it seems that this new conformation may somehow be closely related or resemble the Z-type of conformation provided by Br-DNA. These antibodies do not react with poly(rG).poly(dC) which is known to exist in A-form.

Anti-Br-DNA antibodies showed binding with Z-form of poly(dG-dC).poly(dG-dC). Quantitative precipitin titration data further substantiates the above results. The induced antibodies are thus conformational specific, reacting only with the modified conformation of nDNA.

The importance of reactive groups in the side chains of immunoglobulins in maintaining the native conformation and immuno-chemical reactivity has been and is a broad subject of study (Yasushi et al, 1972). Chemical modification of proteins by virtue of covalent introduction of new groups (without rupture of primary chemical bonds) may modify the intramolecular forces, so as to cause "reorganization of the molecular conformation". The magnitude of this reorganization depends on the characteristics of the groups introduced. This conformational reorganization through its effects on the protein molecule may be similar to denaturation. The biological role of lysine residues in several enzymes has been demonstrated by chemical modification techniques. The disrupting influence of lysine modification depends on the folded stability and on how necessary the lysine residues are for the formation of ordered structures. Lysine residues of anti-Br-DNA immune IgG were modified by trinitrophenylation. The results of the present study demonstrate that by increasing the time of modification of lysine residues anti-Br-DNA IgG lost its binding to the
immunogen. The extent of reaction of TNBS with anti-Br-DNA antibodies suggests that most of the lysyl residues are either on the surface of the IgG molecule or accessible to the modifier.

The inhibition of IgG binding to Br-DNA to the extent of 90% with only 50% modification of lysine residues suggests that these residues are equally involved in antigen binding. Anti-Br-DNA IgG contains 77 lysine residues and to pin-point the involvement of specific lysine residue(s) in antigen binding has not been investigated.

In the present study, various normal human sera and sera of patients with SLE were checked for the presence of anti-DNA antibodies and anti-Br-DNA antibodies. Auto-antibodies play a pathogenic role in variety of clinical conditions. While the direct or indirect pathogenic role of autoantibodies has been well established in conditions such as autoimmune hemolytic anemia, systemic lupus erythematosus, rheumatoid arthritis, myasthenia gravis etc. their precise significance in many of the diseases in which they are detected remains the object of controversy (Melissa V. et al, 1989). Systemic lupus erythematosus (SLE) is a prototype autoimmune disease characterized by the formation of a variety of autoantibodies and hypergammaglobulinemia (Tsutomus T. 1985). Anti-double stranded DNA (anti-ds DNA) is generally recognized as the most sensitive and specific autoantibodies in SLE (John B.H. and R.H. Scofield, 1991).

By competition ELISA, anti-DNA autoantibodies were found to be specific for native double stranded DNA with poor reactivity with single stranded polymer. Our results are in full agreement with previous findings that some antibodies react exclusively with denatured DNA, where as others recognize both native and denatured forms and a smaller but important population prefer native over denatured DNA.
The SLE anti-DNA antibodies showed appreciable binding with Z-conformation of calf thymus DNA brominated under high salt as evident from the results of direct binding assay and competition assay. Sera from SLE and other autoimmune diseases have been found to contain antibodies against Z-conformation of poly(dG-dm^5C). poly(dG-dm^5C) and brominated poly(dG-dC).poly(dG-dC) (Sibley et al, 1984); Lafer et al, 1983).

In conclusion, these studies point out the existence of regions in native DNA capable of transition from B -> Z conformation under adverse conditions. Since Z-DNA is highly immunogenic, the antibodies induced against Br-DNA appears to be highly specific for the modified polymer probably recognizing the changed conformation of the modified polymer. The altered polymer showed appreciable binding with human anti-DNA autoantibodies. The possibility of an altered polymer acting as antigen for the production of human autoantibodies could be one of the factors for the pathogenesis of SLE.