ABSTRACT
Reactive oxygen species (ROS) are implicated in the inflammatory, autoimmune, connective tissue disease, systemic lupus erythematosus (SLE), particularly in respect of processes leading to the formation of pathogenic anti-DNA antibodies. Exposure to ROS increases the antigenicity of DNA. These reactive species are generally produced by ionizing radiation, redox-cycling drugs and many carcinogenic chemicals and can cause DNA damage, including single strand breaks and base modifications. Among these radicals, superoxide anion radical and singlet oxygen species are the most abundant. Superoxide is generated \textit{in vivo} by several enzymatic and non-enzymatic pathways in mammalian tissues. Singlet oxygen is not a direct product of the enzymatic reactions, but rather a secondary reaction product and is a potent biological toxin. Both have been implicated in several disease states via alteration of biomolecules. Oxidative damage to cellular DNA can lead to mutations and may, therefore, play an important role in carcinogenesis and other biological processes.

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease involving both humoral and cellular aspects of the innate and acquired immune systems. Lupus is characterized by autoantibodies with a spectrum of specificities that participate in disease pathogenesis. In SLE, antibodies to DNA occur prominently and serve as markers of diagnostic and prognostic significance. The antibodies are predominantly IgG class and bind to conserved sites on the backbone of both single stranded (ss) as well as double-stranded (ds) DNA. Studies have emphasised that this disease is a complex genetic trait with contributions from major histocompatibility complex (MHC) genes and multiple non-MHC genes. The apoptosis genes \textit{Fas} and \textit{Fas} ligand (\textit{Fas} L) are candidate contributory gene in human SLE, as mutations of these genes result in autoimmunity in several murine models of this disease.

In the present study, commercially available calf thymus DNA was purified and fragmented by using micrococcal nuclease and 200 bp fragments were selected for further studies. DNA was modified by singlet oxygen and superoxide anion radicals generated by riboflavin on exposure to UV light. The results indicated single strand breaks and base modifications. The induced modifications in DNA were analyzed by UV and fluorescence spectroscopic techniques. The modified DNA showed hyperchromicity
at 260 nm, as compared to native DNA, thereby, reflecting the change in DNA helix organisation. The Tm of $^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA was found to be 78°C, whereas, native DNA showed a Tm of 86°C. A net decrease of 8°C in the Tm value of $^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA indicates a partial destruction of its secondary structure. The single strand breaks, induced in DNA as a result of modification, were detected by nuclease S1 digestibility assay. The modified DNA fragments were employed in competitive assays to delineate the epitope recognition of induced antibodies against $^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA.

The antigenicity of $^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA was probed by inducing antibodies in rabbits. The repertoire of specificities of induced antibodies were evaluated by direct binding and competition ELISA. The induced antibodies exhibited polyspecificity, a property commonly associated with SLE anti-DNA autoantibodies. Anti-$^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA antibodies showed preferential binding to ROS-DNA.

Twenty four SLE sera were studied for their binding to native and $^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA. Specificity of antigen binding was assessed by inhibition ELISA. In direct binding assay, all SLE autoantibodies bound $^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA in preference to dsDNA. The preference was reiterated by inhibition ELISA. The binding specificity of native and $^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA with SLE anti-DNA autoantibodies was also confirmed by gel retardation assay.

Similarly, cancer sera were screened for the presence of antibodies reactive with native and $^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA. The study consisted of 34 sera from patients with various types of malignancies. Direct binding ELISA showed greater recognition for $^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA as compared to native form. Four sera from breast cancer showed higher recognition of $^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA than native DNA. Five sera from cancer of urinary bladder showed higher reactivity with $^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA. Four sera from cancer of gall bladder also showed higher recognition with $^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA. Three sera from prostrate cancer showed moderate recognition with $^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA, while only one sera showed almost equal inhibition with both native and $^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA. All the sera of lung cancer, having a history of smoking showed higher recognition of $^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA. Among four sera from oral cancer, two showed higher recognition of $^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA, one showed moderate recognition, while one sera showed higher recognition of nDNA as compared to $^{1}\text{O}_2\cdot\text{O}_2^-$ -DNA. Four sera from cancer of head and neck, two from vulva, one serum each from CML and
Hodgkin’s lymphoma showed higher recognition for $^{1}$O$_2$-O$_2^-$-DNA as compared to nDNA. The results indicate that ROS appears to enhance the antigenicity of native DNA, suggesting role of ROS damaged DNA in the production of autoantibodies in cancer patients.

The role of ROS in the development of cancer was further supported by detection of oxidative lesions in DNA isolated from lymphocytes of various cancer patients, using anti-$^{1}$O$_2$-O$_2^-$-DNA IgG as probe. DNA from two patients with lung cancer recognized anti-$^{1}$O$_2$-O$_2^-$-DNA IgG appreciably and inhibited its activity to 64% and 68%. DNA from patients with oral and prostrate carcinoma too inhibited antibody activity appreciably.

In conclusion, singlet oxygen and superoxide anion radical causes damage to DNA and renders it highly immunogenic. Antibodies against $^{1}$O$_2$-O$_2^-$-DNA are polyspecific in nature resembling the antigen binding characteristics of SLE anti-DNA autoantibodies. It is postulated that $^{1}$O$_2$-O$_2^-$-DNA may play a major role in the production of SLE anti-DNA autoantibodies by the modification of DNA (or nucleosomes) thus forming neoantigen(s) resulting in production of autoantibodies. Further the anti-$^{1}$O$_2$-O$_2^-$-DNA IgG raised in experimental animal was able to detect oxidative lesions in DNA of cancer patients.