Abstract
Free radicals, such as superoxide, hydroxyl, nitric oxide and other reactive oxygen species, like hydrogen peroxide, are formed in vivo. Though nitric oxide, a multi-faceted bioregulatory agent produced by many types of cells, is known to play a critical role in both regulatory processes and cell defense, yet it also participates in collateral reactions, leading to DNA damage and cell death in both nitric oxide generating and neighboring cells.

Though many human genetic disease and cancer are thought to arise from deamination of DNA, yet spontaneous (hydrolytic) deamination is extremely unfavorable energetically. Certain toxic agents may catalyze this reaction and increase the incidence of the genetic disorders. One candidate is nitric oxide, a cigarette constituent and environmental pollutant, produced in numerous cell types in amounts that can total to 200 millimoles per day per person. This bioregulatory molecule can accelerate base deamination, DNA strand breaks, DNA-protein cross-linking and cause deamination-induced genetic changes in the living cells along with apoptosis or cells death.

Nitric oxide is an important mediator of the inflammatory response. It is a strong proinflammatory substance that may increase vascular permeability in inflamed tissues. There is an extensive suggestive evidence that nitric oxide can participate as mediator of tissue damage in autoimmune disease. Elevated nitric oxide biosynthesis has been associated with chronic inflammatory autoimmune diseases including rheumatoid arthritis, inflammatory bowel diseases, glomerulonephritis, vasculitis, multiple sclerosis and systemic lupus erythematosus.

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease of unknown etiology characterized by various immunologic disorders, including production of autoantibodies, formation of immune complexes, decreased serum complement levels, and lymphocytopenia. The complex and non-organ specific nature of SLE has made it difficult of researchers to unravel the genetic defects and pathogenic mechanisms underlying this disease. One of the hallmarks of this disease both in humans and mice, is the loss of tolerance to nuclear antigens. The dominant presence of antibodies against the exposed conformational epitopes on chromatin strongly suggests that the pathogenic immune response in lupus is driven by chromatin. The demonstration of T cells specific for chromatin components that can drive the antinuclear antibody producing B cells tolerance to both murine and human lupus further illustrates how loss of B cell and T cell tolerance to chromatin
constitutes a central feature of lupus. Although antibodies that react with B conformations of DNA are found in sera of patients with SLE, B-DNA per se is a poor immunogen. On the other hand, various other conformations like right handed A-form and left handed Z-form are immunogenic. Also DNA modified with ROS, certain chemicals, in conjunction with the female sex hormone estradiol have been found to result in the production of autoantibodies. It has been reported that ROS modification of dsDNA results in an increased binding of anti-DNA antibodies from SLE sera.

Nitric oxide is known to cause cell death via apoptosis and serum nitrite levels are also reported to be elevated in SLE patients as well. Further, in SLE patients increased apoptosis of circulating lymphocytes has been reported. This increased rate of apoptosis could lead to an overflow of the apoptotic cell material and thus increase nuclear components both normal and modified e.g. dsDNA, nucleosomes or histone in serum of these patients. In addition, dysregulated apoptosis and/or phagocytosis may trigger and provide survival signals for autoreactive B cells.

In the present study, chromatin was isolated from goat liver and modified by nitric oxide radical, generated by reduction of sodium nitrite with sodium dithionite. The nitric oxide induced modifications in chromatin were analyzed by UV spectroscopic, fluorescence, CD and thermal denaturation studies. NO-chromatin showed hyperchromicity at 260 nm as compared to native chromatin, reflecting the presence of strand breaks and alterations in the structure of chromatin. While fluorescence studies showed formation of single strand breaks in DNA and exposure of protein chromophoric groups in chromatin. A net decrease of 9 °C in Tm value of NO-chromatin as compared to native chromatin at 260 nm was observed while Tm value of NO-chromatin was found to be high as compared to native chromatin at 280 nm. This indicates the formation of single strand breaks in DNA and protein-protein/protein-DNA adduct or cross-linking due to nitric oxide modification. The single strand breaks induced in the modified chromatin upon nitric oxide exposure was further ascertained by nuclease S1 sensitivity assay. DNA base modifications in modified chromatin were analyzed by DEAE Sephadex A-25 column chromatography. Guanine was maximally modified followed by cytosine.

Native chromatin and NO-chromatin were used to induce antibodies in rabbits and were found to be immunogenic inducing high titre antibodies. The modified chromatin was highly immunogenic as compared to native chromatin. The repertoire
of specificities of induced antibodies was evaluated by direct binding and competition ELISA. The immunogen showed a high degree of specificity for the induced antibodies. The induced antibodies exhibited polyspecificity, a property commonly associated with SLE autoantibodies. Anti-NO-chromatin antibodies recognized human blood protein, modified tyrosine polymers, nucleosomes and histone.

A group of SLE sera was investigated for the evaluation of anti-DNA autoantibody titre. SLE IgGs isolated from sera of ten patients showing high titre anti-DNA autoantibodies were analyzed for their specificity towards native calf thymus DNA, native chromatin and NO-chromatin by inhibition ELISA. The binding specificity of SLE IgGs with NO modified and native chromatin was further analyzed by gel retardation assay. Direct binding ELISA results show preferential binding of SLE autoantibodies to NO-chromatin in comparison to native chromatin and calf thymus DNA. Inhibition ELISA reiterates the direct binding results. Gel retardation assay further substantiated the binding of native and modified chromatin with SLE autoantibodies. The antigen binding characteristics of SLE IgGs obtained from different patients were remarkably similar, including their binding to native and NO-chromatin. The result suggests that the nitric oxide modification of chromatin causes perturbations resulting in the generation of neo-epitopes, thus making it a potential immunogen.

In conclusion, the nitric oxide radical causes extensive damage to chromatin and renders it immunogenic. The antibodies against modified chromatin are polyspecific in nature, resembling the antigen binding characteristics of SLE autoantibodies. The possibility of nitric oxide modified chromatin in the production of SLE autoantibodies has been suggested.