Introduction
All the systems maintain and transfer their genetic information through a thread-like macromolecular structure known as deoxyribonucleic acid or DNA. The basic structure of DNA was elucidated by Watson and Crick in 1953 which has had the way to understanding gene function in molecular terms. The Watson and Crick B-helix is the most accepted model in solution. The structure of nucleic acids, in solution, cannot be directly predicted from their crystal or fiber structures since it depends on the variation of environmental condition such as solvent, pH, temperature and ionic strength. (Fukudome et al., 1990; Zhong and Johnson, 1990). Since DNA is made up of ordinary molecules that are not endowed with any peculiar kind of quantum mechanical stability, it can attain different conformations such as A-and C-DNA, within the right handed family (Adams 1996) or Z-DNA, which is a left handed double helix, so called because the phosphates in the backbone are zig-zagged and have alternating purine and pyrimidine. It has been demonstrated that deoxyguanosine moieties play an important role in producing left handed Z-structures in poly (dG-dC) (Wang et al, 1979) while the deoxyguanosine residues are responsible for the poly (dA).poly(dT) conformational transitions (Nakamura et al., 1985). A triple helical form called H-DNA has also been reported (Frank-Kamentski, 1990). The various conformations of DNA are in equilibrium with each other (Dickerson et al., 1982; Rich et al., 1984; Pechenaya, 1993). The change in conformation of DNA from B → Z is one of the examples of DNA polymorphism (Pietrasanta et al., 1994). Among the different conformations of DNA, B and Z type do occur in vivo but majority is in the B-form (Jaworski et al., 1987; Drew et al., 1988). A-DNA is usually formed when relative humidity is below 75% i.e. dehydration favours B→A transition (Table 1). Also the conformation of poly(dG), poly(dG) in solution is A-like (Sarma et al., 1986), the dormant spores of Bacillus harbour A-DNA (Setlow, 1992). As said earlier, DNA is highly flexible macromolecule and therefore capable of interacting with other macromolecules and attaining different conformations. DNA undergoing transcription forms DNA-RNA hybrids and double helical RNA hair pins, which have mostly A-DNA type structure (Travers, 1989.)

**Immunogenicity of DNA**

Immunogenicity is not an intrinsic property of an antigen but rather depends on a number of properties of the particular biological system that an antigen encounters.
TABLE 1
Different Conformations of Right Handed DNA

<table>
<thead>
<tr>
<th>FORM</th>
<th>PITCH (Å)</th>
<th>RESIDUE PER TURN</th>
<th>INCLINATION OF BASE PAIR FROM HORIZONTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Na – salt, 75% relative humidity)</td>
<td>28</td>
<td>11</td>
<td>20°</td>
</tr>
<tr>
<td>B (Na – salt, 92% relative humidity)</td>
<td>34</td>
<td>10</td>
<td>0°</td>
</tr>
<tr>
<td>C (Li – salt, 66% relative humidity)</td>
<td>31</td>
<td>9.3</td>
<td>6°</td>
</tr>
<tr>
<td>RNA-DNA hybrid</td>
<td>28</td>
<td>11</td>
<td>20°</td>
</tr>
</tbody>
</table>

Source: Adams et al. (1981)
Immunogenicity is mainly determined by four properties of a molecule namely (i) its foreignness (ii) molecular size (iii) chemical complexity and (iv) ability to be processed and presented with an MHC molecule on the surface of an antigen presenting cell or altered self cell. The antigenic properties of nucleic acid were recognized after finding circulating immune complexes in the sera of patients with systemic lupus erythematosus (SLE) in which native DNA was found to bind with SLE autoantibodies. The anti-DNA antibodies found in SLE sera are known to be polyspecific and have been shown to recognize epitopes that occur not only on differing nucleic acids (Andrezewski et al., 1991) but also on phospholipids (Tiikainem et al., 1991), proteins (Tsuzaka et al., 1996), polysaccharides (Kashihara et al., 1993) and cell membrane structures (Jacob et al., 1986). This polyspecificity / polyreactivity of anti DNA autoantibodies could be due to variable binding sites in individual immunoglobulin or to recurrent epitopes in different antigens. The polyreactivity of anti-DNA autoantibodies suggest that DNA itself may not be the immunogen responsible for anti-DNA antibodies. To study this enigma several experiments were conducted to account for the immunogenicity of DNA. Attempts to induce antibody formation by injection of free nucleic acid or nucleic acid with Freunds adjuvant but without any carrier have led to variable results. Immunization of rabbits with calf thymus DNA did not elicit antibody response (Haskowa et al., 1959, Wilson et al., 1965). Also, they got negative results on injection of purified native or denatured salmon, calf thymus, phage T₄ or E.coli DNA. Later it was found that immunization with a mixture of denatured DNA and methylated bovine serum albumin (MBSA), with or without adjuvant, lead to the formation of antibodies that reacted with denatured DNA but not with native DNA (Plescia et al., 1964). This enhancement of immunogenicity of nucleic acid could be due to the fact that MBSA acts as a carrier that allows its delivery to appropriate cell for antibody formation and also it may protect DNA from degradation by nucleases. Immunization of rabbits with right handed poly(dG-dC).poly(dG-dC) triggered the immune response leading to the production of antibodies that were specific for the polymer but failed to react with either denatured DNA or calf thymus DNA (Lafer and Stollar, 1984; Zarling et al., 1984). Thus it could be said that complexes of double helical polynucleotides with MBSA were effective immunogens that differ from DNA in some aspects of B-conformation. Besides these, DNA complexes with a synthetic immunogenic peptide, Fus-1, induces an anti-double stranded DNA response in
mice (Desai et al., 1993). This led to the conclusion that antigen drive in lupus involves either a substance other than native DNA or DNA in a form (e.g. nucleosome), that is not readily mimicked by artificial complexes (Burlingame et al, 1993; Mohan et al, 1993). Besides these, DNA modified with hormones, drugs and ROS have also been reported to induce antibodies (Moinuddin and Ali, 1994; Arjumand et al., 1995; Ashok and Ali, 1998). Certain nucleic acid polymers like poly(dT), poly(dC), poly(dA), poly(dl) and poly(dG) can induce antibodies that react selectively with the immunogen (Garg and Ali, 1998). It has been reported that naturally occurring human anti-DNA autoantibodies bind to β-estradiol and native DNA suggesting that female sex hormone plays a role in the etiopathogenesis of SLE (Moinuddin et al., 1998). Besides this, there is compelling evidence that bacterial DNA, in contrast to mammalian DNA, can induce variety of responses in both normal humans and animals (Pisetsky, 1996). Contrary to prevailing Dogma that anti-DNA antibodies occur only in SLE, the normal human sera showed significant binding to DNA from two bacterial species, *Micrococcus lysodecticus* and *staphylococcus epidermidis*. Bacterial DNA in contrast to mammalian DNA induces variety of responses in humans as well as in animals. This may be due to the differences between two DNA, bacterial DNA contain sequences that are rarely found in mammalian DNA. Prokaryotic DNA contains a high frequency of methylated adenosine residue which are much less abundant in mammalian DNA (Pisetsky et al., 1989). These methylated bases promote unique conformations. Mammalian DNA in contrast contains methylated C in CpG doublets to much greater extent than bacterial DNA. This is especially relevant because poly (CG) with methylated C is much less mitogenic than poly (CG) with unmethylated C. It is well known that CG rich DNA has tendency to form Z-DNA which is highly immunogenic. However, methylation of C residues hinders the formation of Z-conformation (Messina et al., 1993). Therefore unmethylated CpG sequences are especially mitogenic and immunogenic. Reduced methylation of the C's in CpG either due to failure to methylate C's in certain genes or due to demethylation of CpG's as a result of gene activation results in generation of immunogenic DNA in SLE. Studies have shown and inferred that normal human sera display high titers of antibodies that bind to antigenic determinants on certain bacterial DNA. The antibodies in these sera are highly specific for the different bacterial DNA antigen (Karounos et al., 1988), in contrast to SLE sera which recognize determinants widely present on both
mammalian and bacterial DNA. An interesting observation is that antibodies to bacterial DNA in normal human sera are predominantly IgG2 type whereas lupus anti-DNA antibodies are mainly IgG1 and IgG3 type (Gilkeson et al., 1991). These different IgG subclasses are encoded by different genes hence it can be said that the two DNA acting as antigens activate different germ line CH genes. Among these subtypes IgG1 and IgG3 readily cross placenta and are effective complement activators, they bind with high affinity to FC receptor on phagocytic cells and thus mediate opsonization. Whereas, IgG2 cannot cross placenta and is less effective complement activator. Therefore, it can be said that some bacterial DNA are immunogenic and capable, during host encounter with microorganisms, of inducing antibodies to unique sites on these molecules (Grudier et al., 1998). Further, chemically or physically modified DNA, or helical structures that differ significantly from the B-DNA helix are much stronger immunogenic stimuli than native DNA and most of the antibodies induced by modified DNA do not react or react weakly with the unmodified DNA.

Free Radicals

Free radical is a chemical species formed by homolytic fission of a covalent bond, or by the loss or addition of a single electron from or to a normal molecule. Free radicals possess an unpaired electron in outer shell of the molecule which accounts for their short half lives and high reactivity. In order to attain stability they attack the nearby molecule of opposite spin and captures its electron which results in beginning of a chain reaction such that the attacked molecule becomes a free radical and the process continues. Finally leading to cellular damage (Martínez- Cayuela, 1995). The presence of free radical and their role in health and disease has widely been accepted into the biochemical and medical orthodoxy. The broad definition of free radical includes the hydrogen atom (one unpaired electron), most transition metals and the oxygen molecule itself. It is now well established that free radicals and other reactive oxygen species (ROS) along with reactive nitrogen species (RNS) are continuously produced in vivo, and can damage most cellular components. Many toxic agents also generate intracellular oxygen radicals. In consequence, several antioxidant defence systems limit their damaging effects and also repair systems to prevent the accumulation of oxidatively-damaged molecules (Sies, 1991).
The production of free radical can either be accidental or deliberate; they are generally produced in cells by electron transfer reactions mediated by the action of enzymes or through the redox chemistry of transition metal ions. Some enzymes utilize a free radical at their active site in the process of catalysis, for example ribonucleotide reductase (Richard and Ehrenberg, 1983). Activated phagocytes also deliberately generate superoxide as part of their bactericidal role (Babior, 1978). The major source of free radicals in a cell is electron leakage from electron transport chains, such as those in mitochondria and in the endoplasmic reticulum, to molecular oxygen, generating superoxide. Flavin oxidases located in the peroxysomes also produce superoxide or hydrogen peroxide. The term reactive oxygen species includes not only oxygen-centered radicals as superoxide (O$_2^-$) and hydroxyl ion (OH$^-$), but some non-radical derivatives of oxygen, such as hydrogen peroxide (H$_2$O$_2$), singlet oxygen (O$_2^*$), Hypochlorous acid (HOCI) and ozone (O$_3$) that are involved in oxygen radical production (Weser et al., 1990). The term reactive nitrogen species includes nitric oxide (NO), nitrogen dioxide (NO$_2$), nitrous anhydride (N$_2$O$_3$), peroxynitrite (ONOO$^-$), nitrogen trioxide (NO$_3^-$), nitroxyl anion (NO$^-$) etc.

**Nitric Oxide Biochemistry**

Biosynthesis of nitric oxide takes place by the conversion of L-arginine to L-citrulline (Fig. 1) catalyzed by nitric oxide synthases. The enzyme has three isoforms: neuronal nitric oxide synthase (NOS$_1$), inducible nitric oxide synthase (NOS$_2$) and endothelial nitric oxide synthase (NOS$_3$). Amongst these, the first isoform purified and cloned is neuronal nitric oxide synthase (David et al., 1998) Constitutive NOS is calcium dependent and continuously present whereas inducible nitric oxide synthase is calcium independent and is expressed only after cytokine exposure. Based on this category nNOS and eNOS are constitutively expressed and require elevated levels of Ca$^{+2}$ along with activation of calmodulin to produce NO for brief period of time (Bredt et al., 1992).

**Inducible NOS**

This type II NOS is induced by inflammatory stimuli e.g. cytokines or lipopolysaccharides and is mainly expressed in macrophages. Inducible NOS possess, tightly bound calmodulin. It is also reported that iNOS is not only present in activated...
Enzymatic synthesis of nitric oxide
macrophages but its synthesis can be induced in glial cells, liver and cardiac muscle (Gordge, 1998).

**Endothelial NOS**

It is constitutively expressed in endothelial lining of blood vessels and depends on Ca$^{2+}$. The NO produced by eNOS diffuses into smooth muscle cell of blood vessel and elicits cGMP dependent smooth muscle relaxation and thus increasing blood flow (Nathan et al., 1994).

**Neuronal NOS**

Neuronal nitric oxide synthase is constitutively expressed in post synaptic terminals of neurons and is Ca$^{2+}$ dependent. It is activated by Ca$^{2+}$ influxes caused by binding of neurotransmitter, glutamate, to the receptor in cell membrane, nNOS is also activated by membrane depolarization through opening of voltage gated Ca$^{2+}$ channels (Silvia et al., 1999).

Nitric oxide synthases utilize L-arginine, NADPH and oxygen as a substrate to produce nitric oxide. It is only heme iron dependent tetrahydrobiopterin H$_4$-biopterin enzyme where H$_4$ binds far away and on the wrong site of porphyryin to act as hydroxylating co-factor at distal side of heme iron center (Silvia et al 1999). Other enzymes that use H$_4$ biopterin are e.g. phenyl monooxygenase, tyrosine 3-monooxygenase and tryptophan 5-monooxygenase. In these cases H$_4$-biopterin is directly involved in hydroxylation of substrate, getting oxidized to H$_2$-biopterin, which is again recycled to H$_4$-biopterin by dihydropteridine reductase. Nitric oxide synthase enzyme in its active form is a homodimer where each subunit is composed of C-terminal reductase and N-terminal oxygenase domain (Fig. 2 ). It is also reported that the isolated oxygenase domain remain homodimamic whereas isolated reductase domain is monomeric indicating that the two subunits are joined by their oxygenase domains (Tamir et al., 1996). The oxygenase domain contains a heme group and one binding site for pteridine cofactor tetrahydrobiopterin and L-arginine whereas reductase domain of nitric oxide synthase has binding sites for one molecule of FMN, FAD, and NADPH. Between the reductase and oxygenase domain there is a binding site for calmodulin (Nathan et al., 1994). In case of nNOS and eNOS only Ca-calmodulin complex can activate the enzyme whereas in case of iNOS it is already bound to calmodulin and is fully active. Calmodulin is reported to improve electron flow from NADPH to flavins
Fig. 2. Structure of nitric oxide synthase enzyme.
and also facilitate electron transfer from FMN to heme. Table-2 summarizes some of the properties of nitric oxide synthase enzyme.

Nitric oxide (NO) is composed of an atom of nitrogen and oxygen such that seven electrons from nitrogen and eight electrons from oxygen are involved to form an uncharged molecule (N=O). The high reactivity of nitric oxide is not due to the fact that it contains an unpaired electron rather NO reacts only with those biological molecules that have unpaired orbital electrons e.g. other free radicals or transition metal ions (Padmaja et al., 1993).

The biological reactions of nitric oxide can be divided into three main pathways:

(i) **Diffusion**

Nitric oxide trespasses the cell membrane by simple diffusion and reacts with cellular components. Once inside the cell, it may react with non-heme iron or quench tyrosyl radical of ribonucleotide reductase which may lead to inhibition of DNA synthesis (Roy et al., 1995).

(ii) **Autooxidation to form nitrous anhydride**

Nitric oxide combines with nitrogen dioxide to form nitrous anhydride.

\[
\text{NO} + \text{NO}_2 \rightarrow \text{N}_2\text{O}_3
\]

(iii) **Reaction with super oxide to form peroxynitrite**

Reaction of nitric oxide with superoxide in biological media yields peroxynitrite which is not a free radical but a negatively charged species capable of reacting with almost all biological molecules and hence, a potent oxidant. One of the known fastest reactions of peroxynitrite is its combination with CO\textsubscript{2} to form nitrosoperoxycarbonate adduct.

\[
\text{ONOO}^- + \text{CO}_2 \quad \rightarrow \quad [\text{ONO}_2^- \cdot \text{CO}_2^-] \quad \rightarrow \quad \text{NO}_3^- + \text{CO}_2
\]

Nitric oxide also reacts with molecular oxygen to form NO\textsubscript{2} in gaseous phase whereas in aqueous phase NO and NO\textsubscript{3}\textsuperscript{−} are the final products (Goldstein et al.; 1995).

\[
2\text{NO} + \text{O}_2 \quad \xrightarrow{\text{gaseous}} \quad 2\text{NO}_2 \quad \xrightarrow{\text{aqueous}} \quad \text{NO}^- + \text{NO}_3^- 
\]

The nitroxyl anion (NO\textsuperscript{−}) formed is also known as endothelium derived relaxing factor. NO can also directly interact with hypervalent complexes formed by agents such as H\textsubscript{2}O\textsubscript{2} and reduce it to a lower valency state thus protecting tissue damage (Puppo et
### TABLE 2

**Properties of NOS Enzymes**

<table>
<thead>
<tr>
<th>FORM OF ENZYME</th>
<th>M.WT. KDa</th>
<th>Ca(^{2+}) DEPENDENCY</th>
<th>TYPE OF EXPRESSION</th>
<th>TISSUE EXPRESSION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inducible</td>
<td>130</td>
<td>Independent</td>
<td>Induced by inflammatory stimuli</td>
<td>Macrophages, astrocytes, hepatocytes, smooth muscle cells, epithelial cells.</td>
</tr>
<tr>
<td>Neuronal</td>
<td>160</td>
<td>Dependent</td>
<td>Constitutively expressed</td>
<td>Neuronal and skeletal muscle cells.</td>
</tr>
<tr>
<td>Endothelial</td>
<td>134</td>
<td>Dependent</td>
<td>Constitutively expressed</td>
<td>Endothelial cells and cardiomyocytes</td>
</tr>
</tbody>
</table>
al., 1998; Kanner et al., 1991). NO is also capable of reacting with oxyhemoglobin and results in the formation of met-Hb and NO$_3^-$ (Albina et al., 1993).

\[
\text{Hb} - \text{Fe}^{II} \left( O_2 \right) + \text{NO} \rightarrow \text{Hb-Fe}^{III} + \text{NO}_3^-
\]

Nitric oxide indirectly interacts with biological molecules through the formation of a powerful oxidant i.e. peroxynitrite, which is governed by relative amounts of NO and super oxide produced.

\[
\text{NO} + \text{O}_2^- \rightarrow \text{ONOO}^-
\]

An intracellular source of ONOO$^-$ is mitochondria where aerobic respiration results in the production of superoxide as well as NO. The peroxynitrite formed can also influence protein and enzyme function. This occurs by nitration of tyrosine residues thus contributing to pathological dysfunction (Silvia et al., 1999). The formation of 3 nitrotyrosine is contributed by many nitrogen oxide species such as peroxynitrite, nitrogen dioxide, nitrous acid, nitronium ion etc. This nitrated product is found in many disease states as e.g. chronic inflammation, atherosclerosis, lung injury etc (Beckman et al., 1993; Beckman et al., 1994).

Peroxynitrite (ONOO$^-$), a short lived specie with $t_{1/2} < 1$ sec, is capable of inflicting oxidative damage to a wide range of biological molecules viz., nucleic acids, lipids, thiols etc (Beckman et al., 1996). At pH 6.8 its conjugate acid ONOOH can diffuse through membranes and cause damage at a distance from its site of synthesis.

**Role of Nitric Oxide in DNA Damage**

Nitric oxide mediates DNA damage by three mechanisms:

(a) Formation of nitrosoamines.

(b) Inhibition of DNA lesion repair systems which is also mediated by other genotoxic systems.

(c) Modification of DNA by oxidation products of NO.

There are many carcinogens in human food and drink and can also arise from the normal metabolism of living organisms (e.g. aflatoxins), from cooking process (e.g. in barbecued meat). The most important human carcinogens may be metabolites of gases in the air around us i.e. both nitrogen and oxygen are procarcinogens. Nitrogen is taken into body as nitrates, nitrites, peptides, proteins, and amino acids and its metabolites include higher oxides of nitrogen and peroxynitrite. Although NO is a free radical, it is probably
insufficiently reactive to attack DNA directly. By contrast N2O3, HNO2, ONOO− can nitrate and deaminate DNA and cause strand breaks and mutations (Ames et al., 1997). e.g. ONOO− can induce G → T transversions where as demination of adenine to hypoxanthine can result in transitions AT → GC since hypoxanthine can pair with cytosine. Living organisms have therefore evolved enzymes that can remove deamination products of cytosine (uracil), adenine (hypoxanthine) and guanine (xanthine) from DNA to decrease the risk of mutagenecity (Savva et al., 1995). Estimates of total daily production of oxides of nitrogen in the healthy human body are about 1 mmole/day based on steady state levels of plasma NO3− and NO2− in subjects placed on diet free of these substances (Wennmalm, et al., 1994). Excess production of reactive nitrogen species e.g. as a result of H.pylori infection, chronic inflammation, or excessive consumption of NO2− rich foods may enhance the risk of gastric cancer by mechanisms involving formation of N-nitroso compounds and possibly also deamination of DNA (Oshima et al., 1981; Oshima et al., 1994; Oshima et al., 2003). Deamination and nitration of purine bases has been represented in (Fig. 3 ). Endogenous generation of reactive oxidants has been known to react with DNA (Marnett et al., 2003).

It has also been reported that quinone derivative of catechol estrogen, which is produced by NO mediated oxidation may form covalent adducts with nucleophilic groups of DNA (Dwivedy et al., 1992). Also the catechol-estrogen adducts or complexes are oxidized to quinones which can reduce oxygen to generate O2− ion or in presence of NO releasing compound leads to the production of ONOO− (Yumiko et al., 1998).

Similarly polyhydroxy aromatic compounds such as pyrogallol and 1,4-hydroquinone auto-oxidize easily to form semiquinone radicals that react with dioxygen to generate O2− which in combination with NO releasing compound like SNP, DEA-NO, SPER-NO results in production of ONOO− responsible for DNA damage (Fig. 4) (Yumiko et al., 1997). It is also reported that when cells were exposed to NO it resulted in DNA single strand breakage. However when purified DNA was exposed to NO at concentrations as high as 1.0 M, single strand breaks were not observed. Nitric oxide is also known to protect DNA against chemistry of oxidative stress by inhibiting Fentons reactions of hydrogen peroxide which leads to single strand generation (Pacelli et al., 1994). Some products of oxygen reduction such as superoxide (O2−) and hydrogen peroxide (H2O2) are not capable of reacting directly with DNA except at levels above
Fig. 3. Nitration and deamination of bases.
Fig. 4. DNA damage by peroxynitrite.
their physiological range. Singlet states of oxygen ($^1\text{O}_2$) readily oxidize guanine, the most oxidizable base in DNA. Hydroxyl radical generates multiplicity of products from all four DNA bases e.g. OH can attack guanine at several positions and attack at position 8 generates 8-hydroxyguanine which has many fates depending upon the environment of redox potential (Fuciarelli et al., 1990). Oxidation generates 8-hydroxy guanine whereas reduction leads to a ring opened product as depicted in (Fig. 5). Other reactive species cause different patterns of damage to DNA. This has been established for singlet oxygen (Ravanat et al., 1995; Epe, 1992; Mori et al., 1998), peroxynitrite (Yermilov et al., 1995), Hypochlorous acid (Whiteman et al., 1997) and nitril chloride (Loeb et al., 1989). Hence it is possible to use base damage pattern observed in DNA that has been isolated from cells and tissues in order to gain information about which reactive species may have caused DNA damage in vivo e.g. DNA isolated from brains of patients with senile dementia of the Alzheimer type shows a pattern of purine and pyrimidine damage suggestive of attack by OH whereas in senile dementia of lewy body type elevations in deamination products, suggestive of attack by reactive nitrogen species are observed. By contrast in Parksons disease there is a selective oxidation of guanine ruling out OH as damaging specie (Lyras et al., 1998; Alam et al., 1997).

Direct chemical modification of DNA is only one of several mechanisms by which reactive oxygen/nitrogen/chlorine species can cause mutations or promote carcinogenic states. Oxidation of lipids induced by reactive species can generate end products, such as malondialdehyde and unsaturated aldehydes that can bind to DNA to generate potentially mutagenic lesions. DNA polymerase and repair enzymes could be damaged by reactive species, decreasing the fidelity of replication and slowing the repair of lesions (Chaudhary et al., 1994). Attack on DNA by species such as OH, ONOO', NO$_2$Cl and HOCl produces a multiplicity of products. It is also reported that when 2'-deoxyguanosine was treated with NO/ O$_2$ mixture at pH 7.0-7.8 a novel lesion, N$_2$-nitro-2-deoxyguanosine was detected through RP-HPLC (Yamada et al., 2000). For the last three species, 8-OHdG is not one of the major products formed (Douki et al., 1995; Spencer et al., 1995). Indeed ONOO' and HOCl can destroy preformed 8-OHdG in DNA since hydroxylated guanines are more easily oxidizable than guanine itself. Thus it can be said that 8-OH-dG levels are not always a quantitative index of oxidative attack of
Fig. 5. Generation of 8-hydroxyguanine.
guanine residues in DNA. They can be affected by redox state of the cell and the mixture of reactive species present.

**Defence Against Reactive Nitrogen Species**

It is now well established that reactive species of nitrogen *in vivo* and *in vitro* can damage cells, tissues, proteins, carbohydrates, lipids and DNA. The deleterious reactions of RNOS are partially controlled by antioxidants. There is a good evidence that endogenous antioxidants synthesized by aerobes e.g. SOD, catalase, GSH do not completely prevent damage by reactive species in vivo. Hence efficient repair systems are needed. Repair of the damage done to DNA by reactive species is particularly important as the constant assault by these species on DNA throughout long life span may contribute to age related cancers (Floyd, 1990; Kasai, 1997).

(a) Physical restriction- Selective compartmentalization and physical restriction are important features of defence against deleterious effects of free radical e.g. the free radical intermediate produced in the electron transport chain are tightly coupled in the membrane bound enzymes and carriers. Thus protecting membrane polyunsaturated fatty acids from lipid peroxidation (Williams, 1985)

(b) Control of super oxide-The enzyme super oxide dismutase (SOD) catalyses dismutation of $O_2^-$ to $H_2O_2$.

\[
O_2^- + O_2^- \xrightarrow{SOD} H_2O_2 + ^1O_2
\]

$O_2^-$ is responsible for the production of ONOO$^-$ in presence of NO. This ONOO$^-$ is a potent oxidant and strong DNA damaging and mutating agent. SOD enzymes are metalloproteins, cytosolic SOD are Zn-Cu based enzyme whereas mitochondrial SOD is magnesium based (Singh et al., 1994).

Humans also obtain certain antioxidants from the diet e.g. vitamin E (α-tocopherol) which remove free radicals by reacting directly with them non enzymatically (Som et al., 1983). Some of these antioxidants can be made to exert prooxidant effect *in vivo*, often by interaction with transition metal ions. This is well known that ascorbate in presence of iron salts induce lipid peroxidation, phenols are also known to produce prooxidant effects *in vitro* (Rahman et al., 1989). Antioxidants may have protective actions not only after their absorption into the body, but even before that. The gastrointestinal
tract e.g. salivary and diet derived NO$_2^-$ presumably react with gastric acid to produce HNO$_2$ which decomposes to oxides of nitrogen. Nitrous acid can deaminate DNA bases: guanine is most sensitive, followed by adenine. Several phenolic compounds are powerful inhibitors of deamination, much more than ascorbate (Oldreine et al., 1998). Hence phenols in fruits, vegetables, wines, tea and other beverages could conceivably exert a gastroprotective effect in situations of excess production of reactive nitrogen species e.g. consumption of food rich in NO$_2^-$. Unabsorbed iron and phenolic compounds end up in faeces, since colon is hypoxic; faces incubated under aerobic conditions generate reactive oxygen species at a high rate. The ability of dietary phytates and phenolics to pass unabsorbed through colon where they chelate iron and scavenge reactive oxygen, nitrogen and chlorine species. This may be beneficial in cases where transient rise in intra colonic oxygen tension occurs or inflammation of colon takes place (Babbs, 1990). The conclusions that can be drawn are-:

1. There is no evidence that β-carotene decreases oxidative DNA damage in humans (Von, Poppel, et al., 1995).

2. Vegetables may decrease oxidative DNA damage by mechanisms unrelated to their content of β-carotene or vitamins E and C. (Verhagen et al., 1995).

3. Dietary antioxidants may be beneficial only up to a point.

4. It has been demonstrated that metallothionein inhibits peroxynitrite-induced DNA and lipoprotein damage. Metallothionein functions as an antioxidant that protects against oxidative DNA, protein and lipid damage induced by superoxide anion, hydrogen peroxide, hydroxyl radical and nitric oxide. It was shown that DNA damage by 3-morpholinosydnomine was prevented by metallothionein and SOD where as damage caused by peroxynitrite was prevented by metallothionein only (Cai et al., 2000).

5. Antioxidant and prooxidant activities of flavanoids, which are diphenyl propane derivatives, indicate that they have both deleterious as well as beneficial effects. It has been reported that most of the tested flavonoids except one having orthotrihydroxy group in either the β ring (epigallocatechin gallate) or the A ring (e.g. quercetagetin) act as antioxidants, i.e they inhibit peroxynitrite-mediated formation of 8-nitroguanine in calf thymus DNA (Oshima et al., 1998; Yen and Lai, 2002)
DNA Repair

When cells are exposed to species like reactive oxygen and nitrogen radicals, large spectrum of DNA lesions are produced, therefore, an immediate response to DNA damage is its repair. In mammalian cells these pathways are poorly characterized and despite the abundant information flowing from facile combination of biochemical and genetic approaches in *E.coli* the function of mammalian counterparts of the microbial enzymes is only hypothesized or extrapolated from microbial models. The nucleotide excision repair system of *E.coli* Uvr ABC although not pivotal for handling oxidative damage in contrast to its crucial role in repair of structurally distorting lesions such as pyrimidine photodimers and some carcinogen adducts (Sancar and Tang, 1993) nevertheless, acts as a secondary defence against oxidative damage. On the other hand, recombination mechanisms can in principle repair any DNA lesion provided that an intact copy of the affected region resides in the same cell (West, 1992). Recombination is likely to be crucial for at least two classes of oxidative damages, i.e. interstrand cross links and double strand breaks.

Besides these repair systems, certain enzymes such as DNA glycosylases are important in initiating repair by hydrolyzing the base-sugar (N-C glycosylic) bond of modified or incorrect base to generate a basic apurinic site (Sancar and Sancar, 1988). Endonuclease III of *E.coli* is a thymine glycol glycosylase that specifically cleaves duplex DNA damaged by X-rays, UV light, osmium tetraoxide and acidic pH. Thymine glycol and dihydrothymine were the first identified substrates for the endonuclease III glycosylase (Demple and Linn, 1980). Possible counterparts to *E.coli* endonuclease III have been found in other organisms. Some other glycosylases include formamido pyrimidine glycosylase, hypoxanthine-DNA glycosylase, 5-hydroxymethyl uracil and 5-hydroxymethyl cytosine DNA glycosylase. It has been suggested that the last two glycosylases exist to maintain 5-methyl cytosine in DNA of vertebrate cells in the face of free radical damage. The repair enzymes identified so far can be used as a gentle probe for a more complete understanding of oxidative damage to DNA under physiological conditions.

Autoimmunity

Autoimmune diseases are caused by failure to distinguish between host and foreign antigens. There is no single theory or mechanism that can adequately explain all
features of autoimmune diseases. One theory is to consider the wide spectrum of autoimmune diseases as mosaic of autoimmunity with many pieces.

(i) **T-helper/T-suppressor cell imbalance**

The immune system is controlled mainly by regulatory influence of T-lymphocyte subsets. In normal individuals the ratio of T-helper to T-suppressor in peripheral blood is 2:1 whereas in almost all of the autoimmune disease the ratio increases to 15:1, particularly during active phase of disease. (Raviranjan et al., 2001). In most cases there is significant decrease in T-suppressor cell number and activity which accounts for increased TH/TS ratio.

(ii) **Genetic factors**

Autoimmune disease shows highly significant familial predisposition. The involvement of genetic factors has been linked to the human lymphocyte antigen (HLA) particularly HLA-DR locus. The HLA genes function as secondary genes to allow expression of specific autoantibody or respective disease state (Harley et al., 1998).

(iii) **Hormonal factors**

Autoimmune disease are more common in females than in males with a ratio of 10:1. There is evidence to support the fact that sex related factors are involved in the pathogenesis of SLE (Moinuddin et al., 1998). It is also reported that testosterone and thymic hormones enhance CD8 T cell receptor function (Lahita and Kunkel 1984) whereas estrogen may suppress this function (Talal and Ahmad 1987).

(iv) **Polyclonal B cell activation**

Polyclonal B-cell activation may be one of the mechanisms responsible for over all activation of B-cell and production of autoantibodies in certain autoimmune diseases (Hahn, 1993). B-cell hyper-responsiveness and poly-clonal activation are present in SLE. In autoimmune prone individuals, B-cell are hyper-responsive to polyclonal activators and undergo initial activation followed by expansion including that of the autoreactive clones under the influence of exogenous or endogenous polyclonal activators (Theofilopoulos, 1995).
Classification of Autoimmune Diseases

Autoimmune diseases in humans are broadly divided into two categories.

a) Organ-specific autoimmune disease.
b) Systemic autoimmune diseases.

In organ specific autoimmune diseases the immune response is directed to a target antigen unique to a single organ or gland so that manifestations are largely limited to that gland. Whereas in systemic autoimmune diseases the response is directed towards a broad range of target antigens and involves a number of organs and tissues. These diseases reflect a general defect in immune regulation that results in hyperactive T cells and B-cell. Tissue damage is wide spread (Richard et al., 2000)

Systemic Lupus Erythematosus

Systemic Lupus erythematosus (SLE) is a disease of unknown etiology in which tissues and cells are damaged by pathogenic autoantibodies and immune complexes. Ninety percent cases are in women, usually of child bearing age but children, men and the elderly can also be affected. In the United States the prevalence of SLE in urban areas varies from 15-50 per 100,000 population. It is more common in blacks than in whites. Hispanic and Asian population are also susceptible.

SLE results from tissue damage caused by pathogenic subset of autoantibodies and immune complexes. The abnormal immune response is due to polyclonal and antigen-specific T and B lymphocyte hyperactivity and its inadequate regulation.

Evidence for genetic predisposition includes increased concordance for disease in monozygotic (24 to 58%) compared to dizygotic (0 to 6%) twins. Studies of association, linkage and genome scanning show complex genetic susceptibility (Austin et al., 1999). Most people with homozygous deficiencies of early component of complement (C1q, C2, C4) have SLE or similar disease (accounting for 5% of SLE patients), suggesting that these genes are major predisposing factors. Most patients must inherit multiple susceptibility genes, and probably experience environmental stimuli as well to develop clinical disease. A defective or deleted class III allele, C4AQO, is the most common genetic marker associated with SLE in many ethnic groups (40-50% of patients compared with 15% of healthy controls). One extended haplotype, B8.DR3.DQw2.C4AQO predisposes to SLE in populations with Northern European
heritage. SLE is associated with HLA-DR2 or DR3 in many groups and single-gene association occurs between HLA class II (especially DQ8) and autoantibodies that associate with clinical subsets of lupus (Harley et al., 1998). Genome scanning from several laboratories has shown two regions of chromosome I that link to disease in sibpairs or multiplex families. One region, Iq23 contains the FcγRII A gene; the other Iq41-42, contains poly[ADP-ribosyl polymerase (PARP)] which may be another predisposing gene that plays a role in DNA repair and apoptosis (Austin et al., 1999). Other results of genome scanning suggest that at least 10 other regions on various chromosomes in addition to HLA along with two regions on chromosome I participate in susceptibility (Hahn, 1998).

Environmental factors that cause flares of SLE are largely unknown, with the exception of UV-B (sometimes UV-A). As many as 70% of patients are photosensitive, other factors such as injected alfalfa sprouts, and chemicals, such as hydrazines, have been implicated, searches for viral/retroviral disease inducers have been inconclusive. Although some drugs can induce lupus like disease, there are notable clinical and autoantibody differences between drug induced and spontaneous lupus (Quismoro, 1997).

Abnormal immune responses permit sustained production of pathogenic subsets of autoantibodies and immune complexes. Some autoantibodies, such as anti-DNA can bind to tissue via charge or cross reactivity or in immune complexes, and cause complement-mediated damage (Hahn, 1998). Some anti DNA and anti-RNP antibodies can bind and enter living cells, altering their function. Other autoantibodies cause damage by direct binding to cell membranes (erythrocytes, platelets) that cause those cells to be phagocytized and destroyed. T-cell help is critical to development of full-blown disease, cells of CD4+CD8+, CD4+CD8+ and CD4+CD8+ phenotypes all help autoantibody production in SLE. The structure of antigens that stimulate autoantibodies is under investigation. Some are clearly derived from self antigen like nucleosomes, ribonucleoprotein, erythrocyte and lymphocyte surface (Table 3) Some may be from external environment and mimic self (e.g. components of vesicular stomatitis virus mimic peptides in Sm antigen (Kimberley, 1994). Therefore it can be said that some individuals are genetically predisposed to SLE. Under the influence of multiple genes,
**TABLE 3**

**Principal Antinuclear Antibodies in Systemic Lupus Erythematosus**

<table>
<thead>
<tr>
<th>SPECIFICITY</th>
<th>TARGET ANTIGEN</th>
<th>FUNCTION</th>
<th>FREQUENCY IN SLE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native DNA</td>
<td>dsDNA</td>
<td>Genetic information</td>
<td>40</td>
</tr>
<tr>
<td>Denatured DNA</td>
<td>ss DNA</td>
<td>Genetic information</td>
<td>70</td>
</tr>
<tr>
<td>Histones</td>
<td>H1, H2A, H2B, H3, H4</td>
<td>Nucleosome structure</td>
<td>70</td>
</tr>
<tr>
<td>Sm</td>
<td>Sn RNP proteins, B, B', D, E</td>
<td>Spliceosome component, RNA processing</td>
<td>30</td>
</tr>
<tr>
<td>U1 RNP</td>
<td>Sn RNP proteins, A, C, 70K</td>
<td>Spliceosome component, RNA processing</td>
<td>32</td>
</tr>
<tr>
<td>SS-AR/Ro</td>
<td>60 Kd and 52 Kd proteins, complexed with Y1- Y5 RNA</td>
<td>Unknown</td>
<td>35</td>
</tr>
<tr>
<td>SS-B/La</td>
<td>46 Kd protein complexed with various RNA</td>
<td>Regulation of RNA polymerase-III</td>
<td>15</td>
</tr>
<tr>
<td>Ku</td>
<td>86 Kd and 66 Kd protein</td>
<td>DNA binding</td>
<td>3</td>
</tr>
<tr>
<td>PCNA/Cyclin</td>
<td>36 Kd protein</td>
<td>Auxillary protein of DNA polymerase</td>
<td>10</td>
</tr>
<tr>
<td>Ribosomal RNP</td>
<td>38 Kd, 16 Kd, and 15 Kd phosphoproteins, associated with ribosomes</td>
<td>Protein synthesis</td>
<td>10</td>
</tr>
</tbody>
</table>

*Source: Tan, E.M. (1989)*
possibly triggered by environmental challenges and highly influenced by sex, they may
develop a number of different clinical syndromes that fulfill diagnostic criteria for SLE.

**Clinical Manifestations and Differential Diagnosis**

The presence of characteristic antibodies confirms the diagnosis of SLE, ANAs are the best screening test. If the test substrate contains human nuclei more than 95% of the lupus patients will be positive. Positive ANA test is not specific for SLE (Boumpas et al., 1995). ANAs occur in some normal individuals (usually low titre). The frequency increases with aging, other autoimmune diseases, viral infections, chronic inflammatory process. Moreover, several drugs induce anti-nuclear antibodies. Therefore a positive ANA test supports the diagnosis of SLE but is not specific, the negative test makes diagnosis unlikely but is not impossible. Antibodies to double-stranded DNA (dsDNA) and to Sm are relatively specific for SLE (Hahn, 1998). High serum level of ANAs and anti ds-DNA and low levels of complement usually reflect disease activity, especially in patients with nephritis. Total functional hemolytic complement (CH50) levels are most sensitive measure of complement activation. Very low levels of CH50 with normal levels of C3 suggest inherited deficiency of a complement component, which is highly associated with SLE and with negative ANA (Hahn, 1998). Hematologic abnormalities include anemia, leukopenia, lymphopenia, thrombocytopenia (Boumpas et al., 1995).

Urine analysis should be performed and serum creatinine levels should be measured periodically in patients with SLE. With active nephritis, the urine analysis usually shows proteinuria, hematuria, and cellular and granular casts (Stone et al., 1998).

**Drug Induced Lupus**

Several drugs including procainamide, hydralazine, isoniazid, chlorpromazine, D-penicillamine, practolol, methyldopa, interferon-α etc cause a syndrome like SLE (Schur, 1993). The syndrome is rare with all but procainamide. There is genetic predisposition to drug induced lupus. Partly determined by drug acetylation rates. Procainamide induces ANA in 50 to 75% individuals within a few months, hydralazaine induces ANA in 25 to 30%. Between 10 to 20% of ANA positive individuals develop lupus-like symptoms (Cowchock, 1998). Most common are systemic complaints and arthralgias, polyarthritis and pleuropericarditis. Renal and CNS involvement are rare. All
patients have ANA and most have antibodies to histones. Antibodies to ds DNA and hypocomplementemia are rare (Raeder et al., 1985). This is a helpful point in distinguishing drug induced from idiopathic lupus.

Role of Nitric Oxide in SLE

Nitric oxide (NO) is a mediator of a variety of normal physiologic functions including maintaining vascular tone and also functions as a neurotransmittor. NO modulates the production of Th1 cytokines and, depending on the specific immune cell type, may induce or inhibit apoptosis (Taylor-Robinson et al., 1994). A number of inflammatory mediators in murine models stimulate NO production e.g. lypopolysaccharide, cytokines including interleukin-12, and tumor necrosis factor-α also up regulate iNOS resulting in release of NO. In murine models of lupus, NO is a mediator of disease, as blocking NO production prevents or delays disease manifestations (Oates et al., 1997). In human autoimmune disease, studies using different measures of NO revealed increased levels of NO production in rheumatoid arthritis, ulcerative colitis and to more extent lupus compared to healthy controls. It was reported that expression of iNOS was increased in the kidneys of patients with lupus. Increased levels of serum nitrate/nitrite (N/N metabolites of NO) were found in patients with SLE (Kashem et al., 1996). These results suggest that measure of NO production, although not specific for lupus, may be useful indicators of lupus disease activity in individual patients followed over time. It has been postulated that similar to animal models, monocytes and tissue macrophages are primary cells that over produce NO in lupus and that local production of NO occurs in kidneys by infiltrating macrophages (Gilkeson et al., 1999). Although local NO production appears deleterious in lupus, productions of NO in other disease states may be beneficial. Studies in animal models of multiple sclerosis and inflammatory bowel disease suggest that the local production of NO may be beneficial by inducing apoptosis of inflammation cells and by turning off production of certain cytokines (Mc-Cafferty, 1997). It has also been reported that blocking nitric oxide production rather than after clinical presentation was effective in MRL-Lpr mice. This difference in efficacy between early and late therapy may reflect that:

(a) Once inflammation has initiated, multiple pathways in addition to those mediated by NO are responsible for its progression.
(b) NO is an important mediator in early, acute but not in chronic inflammation.
(c) While reducing NO production may result in reduced inflammation (Oates et al., 1997)

Also to relate the role of NO in SLE its site of production and quantum is of relevance. Patients with SLE showed upregulation of iNOS in normal appearing vascular endothelium. These endothelium also over-express the vascular adhesion molecules ICAM-1, E-Selection and VCAM (Belmont et al., 1994). SLE is a prototypical autoimmune disease in which over production of nitric oxide (NO) has been implicated in its pathogenesis. Many studies have indicated a correlation between serum nitrate and nitrite (NO$_x$) levels and the disease activity. But this measure can be falsely elevated by exogenous dietary and medication sources of NO$_x$. These variables can make NO$_x$ a less reliable tool for studying the role of NO in SLE. Peroxynitrite, a by product of NO and superoxide, nitrates tyrosine moieties. The resulting 3-nitrotyrosine (3NT) serves as a long-term indicator of NO-mediated protein modifications that is not affected by exogenous sources of NO$_x$ or serum thiols (Sampson et al., 1996). It was correlated that for these reasons serum 3NT levels were related to lupus disease activity more significantly than serum NO$_n$. It was found that patients having active lupus nephrites had higher levels of serum 3NT than those without renal disease. Immuno-histochemical analysis of renal biopsies from subjects with active proliferating lupus nephritis revealed normal expression of iNOS Therefore, it could be concluded that over production of NO may play a pathogenic role in SLE and lupus nephrites and that the serum 3NT may be a useful tool for studying contributions of NO to the pathogenesis of SLE (Oates et al., 1999).

**Treatment**

There is no cure for SLE. Therefore treatment is symptomatic and is planned to control acute flares and maintain strategies in which symptoms are suppressed. Basically treatment is done on the basis that-

(a) Patients have mild disease with no life threatening manifestations.
(b) Life threatening, severely disabling manifestations of SLE.

Approximately 25% of patients have mild disease; therefore these patients are managed without glucocorticoids. NSAID (nonsteroidal anti-inflammatory drugs) are
prescribed along with salicylates (Kimberly, 1994) to improve arthralgias, arthritis, myalgias, fever etc. For treating dermatitis and fatigue, antimalarials are used e.g. doses of 400 mg hydroxychloroquinone may improve skin lesions. They are advised for regular ophthalmologic examinations. Other therapies include sunscreens (SPF ≥ 1.5), tropical, intralesional glucocorticoids, vetinoids, dapoones etc may be given. Recent studies indicated the beneficial use of dihydroepiandrosterone to lower disease activity in patients with mild SLE (Ward et al., 1995).

Life threatening and severely disabling manifestations of SLE that are responsive to immunosuppression should be treated with high doses of systemic glucocorticoids (1 to 2 mg/kg/day). When disease is active, glucocorticoids should be given every 8 to 12 hr. After the disease is controlled the dose is tapered as rapidly as the disease permits (Tumlin, 1999). The undesirable effects of chronic glucocorticoid therapy include cashingoid habitus, weight gain, hypertension, infection, hirsutism, osteoporosis, glaucoma, insomnia, psychosis etc.

The uses of cytotoxic agents such as azathioprine, chlorambucial, cyclophosphamide, methotrexate etc are beneficial in controlling active SLE and reducing use of steroidal drugs. For patients with lupus nephritis less renal failure and better survival is maintained by combining cyclophosphamide with glucocorticoids. Among cytotoxic agents cyclophosphamide is most toxic and most effective (Ward et al., 1995). Azathioprine is least toxic and the recommended doses are 2 - 3 mg/kg/day. The undesirable side effects of cytotoxic drugs include bone marrow suppression, increased infection, irreversible ovarian failure, hepatotoxicity, bladder toxicity etc. (Werth et al., 1997).

**Cancer**

Unlike free living cells that compete to survive, the multicellular organisms are committed to collaboration. Any mutation that gives rise to selfish behavior by individual members of the cooperative will jeopardize the future of whole organism. Mutation, competition, and natural selection operating within the population of somatic cells are best ingredients of cancer. It is the disease in which individual mutant cells begin by prospering at the expense of their neighbours but in the end destroy the whole cellular society and die. Cancer cells are defined by two heritable properties.
(1) Reproduce in defiance of the normal restraints
(2) Invade and colonize territories normally reserved for other cells.

Cancers are classified according to the tissue and cell type from which they arise. Cancers arising from epithelial cells are termed as carcinomas. Those arising from connective tissues or muscle cells are termed sarcomas. Cancers derived from hemopoietic cells and from nervous system are termed as leukemia's.

When a cancer has metastasized its origins can usually be traced to a single primary tumor, arising in an identified organ and presumed to be derived by cell division from a single cell that has undergone some heritable change that enables to outgrow its neighbours. One type of demonstration comes from analysis of the cellular DNA. It was found that in almost all patients with chronic myelogenous leukemia e.g. the leukemic white blood cells are distinguished from normal cells by a specific chromosomal abnormality known as Philadelphia chromosome (Fearon et al., 1987).

This Philadelphia chromosome is created by translocation between long arms of chromosome 9 and 22. DNA at the site of translocation when cloned and sequenced, was found to be identical in all the leukemic cells in any given patient, but differs by few hundred or thousand base pairs from one patient to other (Groffen et al., 1984).

Cancer has multifactorial etiology which includes both genetic and environmental factors (Gourley et al., 1992). Among the environmental factors, consumption of tobacco in various forms (e.g. smoking, chewing) is the major cause of cancer of lungs, larynx, mouth, pharynx, bladder, pancreas etc. Alcohol consumption is associated with cancer of esophagus, liver and rectum (Eskelson et al., 1993). Dietary factors such as high fat diet, beef consumption, food additives, and contaminants have also been related to cancer (Ames, 1983).

Studies indicate that most of these exogenous carcinogens act via production of reactive oxygen or reactive nitrogen species. Thus reactive oxygen/nitrogen species produced exogenously as well as endogenously are known to play an important role in the initiation and promotion of multistage carcinogenesis (David et al., 1998, Marletta, 1988).
Role of Reactive Nitrogen Oxide Species in Cancer

Cancer can be defined as malignant transformation of a cell or group of cells, which due to its multifactorial etiology involves both genetic and environmental factors (Hussain et al., 2003). Among the environmental factors the RNOS are generated both in the environment and pathophysiologically in mammals and induce many direct or indirect reactions mediated by NO leading to modulation/damage to biological molecules. Therefore, it can be said that RNOS has both protumor as well as antitumor effect.

Anti Tumor Effect

Nitric oxide (NO) acts as an immune effector generated by iNOS in macrophages, neutrophils etc. In large quantities it kills or inhibits growth of many pathogens and block viral respiration. It also up regulates tumor suppressor p-53 gene (David et al., 1998). NO donors are capable of inhibiting angiogenesis, metastasis and tumor growth. Nitric oxide also inhibits DNA damage mediated by ROS via Fenton’s chemistry besides inhibiting hydroxylation reactions (Miles et al., 1996). NO is reported to have tumor killing activity against many tumors. Since high risk carcinogenic sites are those exhibiting prolonged expression of iNOS during chronic inflammation. NO generated from macrophages, Kpuffer cells and NK cells are capable of inhibiting replication alongwith antitumor effect in the target cells (Kurose et al., 1993; Xio et al., 1995). Murine embryonic liver cells, BNL CL.2 are capable of expressing iNOS in response to IFNγ thus accumulating NO.

In humans few tumors are caused by viruses e.g. liver cancer is caused by hepatitis B-virus, cervical cancer by human papilloma virus, adult T cell leukemia by human T-cell leukemia virus. Interferon γ is particularly important in limiting the spread of certain viral infections and capable of expressing iNOS. Increased concentration of NO in or near the target cell may act as tumorcidal since NO is capable of eliminating intracellular pathogens and blocking viral replication (Pipili-Synetos et al., 1995). Nitric oxide is capable of protecting cell from apoptosis or mediating apoptosis depending upon the cell type. NO protects rat ovarian follicles from atretic generation on one hand while on the other it induces apoptosis in tumor cells like in mastocytoma, sarcoma, melanoma etc. (David et al., 1998; Fadeli et al., 2003). NO is also reported to protect tissue from
peroxide mediated damage by scavenging metallooxospecies which are formed by oxidation of metal species or metal oxygen species by H$_2$O$_2$ (Gorbunov et al., 1995). It has also been reported that animal subjects having tumorous growth acquire the ability through which their tumor tissues suppress the expression of iNOS and thus reduce the concentration of NO (Gardner et al., 1995; Lejeune et al., 1994). Tumor growth is enhanced by accelerated angiogenesis by down regulating the production of vascular endothelial growth factor which is the mediator of angiogenesis. NO is also capable of suppressing metastasis by reducing intracellular stores of GSH or by blocking the adhesion of tumor cells to venular side of microcirculation (Kong et al., 1996). It has also been reported that NO produced in vasculature of brain limits the spread of colon cancer to brain. Liver endothelial cells produce NO which curbs the metastasis of melanoma cells to lungs. Though excessive production of NO is associated with tissue injury, it has been reported that endothelial NO production plays protective role in microvasculature (Claney et al., 1995). NO is also reported to inhibit platelet aggregation and it reduces platelet adhesion to endothelial monolayers. The defensive properties accounting for beneficial effect of NO in IL-2 induced injury include protection against tissue injury in myocardial ischemia reperfusion and adult respiratory distress syndrome (Roissaint et al., 1993; Lefer, 1992). It is also reported that when cells were exposed to NO it resulted in DNA single strand breaks. However, when purified DNA was exposed to NO at concentrations as high as 1.0 M single strand breaks were not observed (Nguyen et al., 1992; Routledge et al., 1993).

**Protumor Effect**

Carcinogenesis can be defined as a malignant transformation of a cell or group of cells. This process can be divided broadly into two stages - initiation and promotion. The initiation phase involves an irreversible modification of the genetic material of the cell caused by single exposure to any carcinogenic agent where as promotion requires multiple exposure to the promoter to alter gene expression and produce a tumor (Lefer, 1992). The reactive oxygen species are generated both physiologically and pathologically in mammals and induce many kind of cellular damage including DNA damage (Ames et al., 1993; Oshima et al., 2003). Since DNA plays a central role in information transfer attention has focussed on oxidative damage as significant source of
mutations that lead to cancer and other human pathologies (Ames, 1989). The possible role of ROS modified human DNA in cancer has been identified from studies in our lab and it has been found that the binding of circulating antibodies in cancer sera was much stronger with ROS-modified DNA than native DNA. The ROS modified DNA has been shown to be a better inhibitor of naturally occurring antibodies in majority of cancer patients than native human DNA (Saba et al., 1999), reiterating the enhanced recognition of ROS-DNA.

Nitric oxide, a paramagnetic diatomic and uncharged molecule, with an unpaired electron and therefore highly reactive radical with half life of 2 to 30 sec. Production of NO has been linked to endogenous carcinogenesis (Silvia et al., 1999). Induction of apoptosis by NO has also been observed in culture of macrophages and pancreatic β-cells. Macrophages exposed to nitric oxide exhibited typical morphology and showed DNA fragmentation indicating apoptotic cell death (Nicotera et al., 1995). NO also induced cell death and showed toxic effects in two different cell lines viz (CHO-AA8) Chinese hamster ovary cells and (TKG) human lymphoblastoid cells, highlighting the role of NO in the onset of mutagenesis and cell death and the involvement of these process in cancer and inflammatory disease (Stopper et al., 1999). NO also showed genotoxic effects by abolishing cell growth which is a valuable parameter sensitive to different kinds of damage e.g. membrane damage, energy depletion, organelle damage and enzyme release. ONOO⁻ is short lived t ½ <1 sec and is capable of oxidative damage of wide range of biological molecules e.g. nucleic acids, lipids, thiols, etc. (Beckman, 1994). At pH 6.8 its conjugate acid ONOOH can diffuse through membranes and cause damage at a distance from its site of synthesis (Marla et al., 1997). Sensitive to apoptosis gene (SAG) protein, a novel Zinc RING finger protein is redox responsive and has been reported to protect mammalian cells from apoptosis. The sulphahydryl group of cystein in SAG directly reacts with peroxynitrite to prevent DNA damage.

It is well known that solid tumors require tumor angiogenesis for their growth i.e. the tumorous cells should be well supplied with oxygen, nutrients and growth factors, besides this an important feature of malignancy is enhanced vascular permeability which is regulated by endothelial cell production of vasoactive substances. The endothelial cells synthesize NO by eNOS which helps in vascular permeability and relaxation (Angard, 1994). Various tumors overexpress NOS (Thomsen et al., 1994; Thomsen et al., 1995). A
study between the relationship of malignancy and eNOS expression in endothelial cells of tumor vessels showed that astiocytic tumor vessels possess higher level of nitric oxide production than do normal vessels and found that there was significant correlation with the proliferative potential and eNOS expression in tumor vessels (Iwata et al., 1999). Several studies have shown that NO and its reactive derivatives i.e ONOO− were found to be elevated in infection and inflammation and plays important role in carcinogenesis (Oshima et al., 1994; Wiseman et al., 1996). It has been found that carcinogenic effect of NO may be due to its cytotoxic potential which lead to reduced cell viability (Stopper et al., 1999).

Oxidative stress mediate DNA damage by the formation of ONOO−. It was confirmed that DNA strand breaks were generated when plasmid DNA was incubated with NO− donor compound and polyhydroxy aromatic compound. On autooxidation polyhydroxy aromatic compound produce O2− (superoxide). Simultaneous generation of O2− and NO leads to formation of ONOO−, similarly catechol-estrogens generate ONOO− resulting in DNA damage (Yumiko et al., 1997).

Nitrosative stress leads to the formation of nitrosamines e.g. RNOS generated from acidic nitrite is potentially carcinogenic in stomach (Correa et al., 1975; Lintas et al., 1982) Nitrosamines are formed under conditions of inflammation which can lead to cancer (Oshima et al., 1994). It has also been demonstrated that sufficient nitrosative stress is present in vivo in some conditions which leads to the formation of nitrosamines that are highly carcinogenic (Liu et al., 1992). NO also enhances tumor production by increasing production of PGE2, a prostaglandin which increases the vasculature and thus angiogenesis (Inano and Onoda, 2003). Furthermore tumor growth is supported by uptake of nutrients (Noguchi et al., 1996). Cells lacking Cu, Zn-SOD are reported to be more susceptible to NO and ONOO− (Lin et al; 1995).

NO inhibits ribonucleotide reductase which results in impaired DNA synthesis as deoxyribonucleotides are no more available (Lepoivre et al., 1994). Since ONOO− causes DNA breaks, this leads to activation of poly(ADP-ribose) polymerase to repair damaged DNA (Zhang et al., 1994). The activated PARP transfers about 100 ADP ribose moieties from NAD+ to nuclear proteins e.g. histones and PARP itself (Szabo et al., 1996). ADP ribose polymers thus formed are degraded by glycohydrolases followed by NAD+ resynthesis which is a futile cycle and depletes ATP (Szabo et al., 1996).
Treatment

Treatment for cancer can be broadly divided into two categories.

(a) **Primary cancer treatment (Surgery and Radiation)**

(a) **Systemic cancer therapy**

(a) Primary cancer treatment - Most cancers present initially are localized tumor nodules and cause local symptoms. Depending upon type of cancer, initial therapy may be directed locally in the form of surgery or radiation. Surgical excision or local radiation (or both) is the treatment of choice for a variety of potentially curable cancers including most gastrointestinal and genitourinary cancers, central nervous system tumors and cancers arising from breast, thyroid or skin along with most sarcomas (Dearnley, 1999).

CT and MRI play an increasingly important role in non-invasive tumor staging (De Whirst, 1997). Based on the results of prospective clinical trials, positron emission tomography (PET) appears to be more sensitive method than traditional method with CT scanning. PET has also been shown to improve detection of recurrent disease for the purpose of second look laprotomy and debulking in colon cancer patients with rising levels of carcinoembryonic antigen (CEA). A monoclonal antibody against CEA labeled with technitium Tc99m (arcitumomab, CEA scan) is now approved for imaging of cancers with increased levels of CEA and has been found to be more sensitive and specific than CT scan (Pieterman 2000). Surgery may also play an important role in the treatment of selected patients with limited metastatic cancer. For certain tumor sites complete surgical removal of tumor can be disfiguring, disabling or unachievable and under these circumstances primary local therapy with ionizing radiation may prove to be the treatment of choice (Paszat, 1998). Well-oxygenated tumors are more radiosensitive than hypoxic tumors since they are bulky, implying a potential synergistic role of surgical debulking prior to radiotherapy. Radiation therapy is normally delivered in fractionated fashion over 4-6 weeks. This method has radio biologic superiority by permitting time for recovery of normal host tissues from sub lethal damage during treatment (Thomas, 1999). For most tumor types, there is a sigmoid curve of increasing rate of control of local tumor with increasing radiation dose. Radio sensitive tumors usually exhibit radio-sensitivity over the dose range of 3500-3000 cGy (Recht, 2001).
Systemic Cancer Therapy- Use of cytotoxic drugs, hormones, antihormones and biologic agents has become a highly specialized and increasingly effective means of treating cancer (Kaye, 1998) while most anticancer drugs are used systematically, there are selected indications for local or regional administration. Regional administration involves direct infusion of active chemotherapeutic agents into the tumor site (e.g. intravesical therapy for bladder cancer, intraperitoneal therapy for ovarian cancer etc. (Childs, 2000). In some instances e.g. Hodgkin's disease, breast cancer, lung cancer, optimal therapy may require a combination of therapeutic resources e.g. radiation plus chemotherapy, rather than either modality alone (Diel, 1998; D'Angelica et al., 1997).

Objective of the Study

Free radicals are produced in living cells both through endogenous as well as exogenous pathways. These may include oxygen as well as nitrogen radical species. Nitrogen free radicals include nitric oxide and its derivatives which are capable of causing DNA damage both through oxidative as well as nitrosative means which could be one of the factors leading to inflammatory diseases, autoantibody production, increased mutagenicity and carcinogenesis.

In the present study human placental DNA is purified free of proteins and single stranded DNA and subjected to modification by the synergistic action of SNP (a NO donor) and Pyrogallol (a polyhydroxy aromatic compound). The modified DNA is characterized by various techniques like Spectroscopy, thermal denaturation studies, simple and alkaline gel electrophoresis and quenching studies. Antibodies against the modified DNA are induced in experimental animals and the binding characteristics and specificity of the induced antibodies is analysed by direct binding and competition ELISA. Possible role of the RNS modified DNA in Cancer and SLE has been probed by studying the binding of the circulating autoantibodies with the modified and native DNA. Anti-RNS-DNA IgG has been used to detect damage in the DNA isolated from different cancer patients. The results have been compared with the binding of induced IgG to the DNA isolated from healthy subjects.