Introduction
The beautiful double helix structure of DNA was discovered in 1953 by James Watson and Francis Crick. DNA contains the instructions to make each one of us. Its digital code is similar to binary, except that instead of two alternatives (designated 0 and 1) it contains four (designated A, C, T and G) (Simon Boa, 1997). It is the position of these four bases in the sequence, which generates the information to make a protein. As well as generating proteins, DNA is also controlled by them. Specific proteins repair it when it gets damaged, tell it when to copy itself and regulate protein production. Some proteins physically interact with the DNA and together they are called “chromatin”. Eukaryotic cells must accomplish the daunting task of packaging an enormous amount of DNA into their nucleus, while ensuring the proper expression of a subset of genes and the silencing of other regions of the genome. Cells accomplish this feat by packaging DNA into chromatin (Rountree et al., 2001). All of the human genome is packaged into chromatin. Although chromatin was historically thought of as an inert repressive structure, we now know that it is truly a living vibrant entity (Wolffe, 2001). Chromatin consists of DNA and associated proteins. There are two types of proteins in chromatin: histones and non-histone chromosomal proteins. Histones are small, well-defined, basic proteins, whereas non-histone chromosomal proteins include a large number of widely diverse structural, enzymatic and regulatory proteins, most of which are yet to be characterized.

During the 1970s, a combination of physical and molecular biology techniques revealed that chromatin consists of a repetitive nucleoprotein complex, the nucleosome (Kornberg, 1974). The laboratory of Pierre Chambon was the first to name the nucleosome (Germond et al., 1975). The nucleosome particle comprises a histone octamer with two copies of each of the histones H2A, H2B, H3 and H4, wrapped by 147 bp of DNA. In the octamer, histones H3 and H4 are assembled in a tetramer, which is flanked by two H2A-H2B dimers. A variable length of DNA completes the second turn around the histone octamer and interacts with a fifth histone, named H1 (Ballestar and Estellar, 2002). The histone octamer wrapped by 147 bp of DNA is called as the nucleosome core particle. The DNA between the nucleosomes is called the linker segment. The linker segment gives unfolded chromatin a beads on a string appearance.
The average linker length is variable in different species, it ranges from zero to a maximum of about 100 base pairs. Wolfe in 1993 suggested that linker lengths tend to be shortest in the lower eukaryotes, intermediate in plants, and longest in higher eukaryotes.

**Antigenicity of chromatin**

Chromatin has been implicated as an important target of autoantibodies in idiopathic and drug-induced lupus for decades, but the antigenicity of chromatin has only recently been dissected (Burlingame and Rubin, 1996). Although anti-DNA antibodies are considered diagnostic of systemic lupus erythematosus (SLE), they are expressed in association with antinuclear antibody specificities, suggesting a role for both generalized as well as antigen specific immune abnormalities in their etiology (Pisetsky, 1992; 1997). The notion that DNA is immunologically inactive, derives primarily from unsuccessful efforts to replicate lupus by immunization of normal animals with DNA (Messina et al., 1993). However DNA complexes with a synthetic immunogenic peptide Fus-I 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 DNA or DNA in the form of nucleosomes. (Burlingame et al., 1993; Mohan et al., 1993).

The nature of autoantigen that initiates the production of anti-double stranded DNA (anti-dsDNA) and anti-histone antibodies in systemic lupus erythematosus has remained obscure. Hardin in 1986 noted that patients with SLE responded primarily to external features of intact nucleosomal segments of chromatin, since the antibodies produced were directed to both histones and dsDNA.

It has been suggested that in SLE, antibodies to individual histones or to dsDNA are minor components of the IgG anti-chromatin (anti-nucleosome) response (Chabre et al., 1995). Several studies have reinforced the concept that SLE is an anti-chromatin disease; e.g., that the autoimmune response is against chromatin with the development of antibodies directed to various epitopes on chromatin including but not limited to DNA (Mohan et al., 1993; Burlingame et al., 1993; 1994; Amoura et al., 1994; Chabre et al., 1995; Tax et al., 1995). The possibility that anti-nucleosomal antibodies may generally be involved in nephritis has also been suggested by experimental and clinical studies.
In a clinical study of SLE, antibodies to epitopes on chromatin were found to be more highly associated with nephritis than antibodies to DNA (Burlingame et al., 1994). The genesis of the immune response to chromatin in MRL/Mp-lpr/lpr mice was investigated (Burlingame et al., 1993). The results demonstrated that many features of anti-chromatin autoantibody production resemble those of an active T cell-dependent immunization process, and that antibodies recognize a wide spectrum of epitopes on chromatin. These findings imply that chromatin is the structure that initiates the production of anti-histone, anti-subnucleosome, and anti-dsDNA autoantibodies. It had been proposed that a defect in the catabolism of chromatin may render it immunogenic. A clear example of altered DNA/chromatin comes from examples of patients with drug-induced lupus. Drugs that induce lupus like side effects, have been proposed to act in different ways – by inhibiting DNA methylation, by inducing a B-DNA to Z-DNA transition, by generating oxidative metabolites that can cause massive DNA-damage resulting in release of altered types of subnucleosomes, or by inactivating complement C4. Some of these mechanisms may generate altered histone or histone/DNA epitopes, thus triggering non-tolerant Th cells (Mohan and Datta, 1995). It has been suggested that the change of chromatin’s antigenicity by environmental factors and genetic background may be the common pathway to SLE pathogenesis (Lu et al., 1998). Exposure of autoimmune (NZBXNZW) F(I) mice to pristane, a model environmental trigger, synergistically activated the production of anti-chromatin/DNA antibodies and dramatically accelerated renal disease (Yoshida et al., 2002). Furthermore, it has been reported that chromatin may be one of the initiating autoantigens and the possibility that nucleosomes may also be an autoantigen in human SLE, is supported by several observations demonstrating the presence of oligonucleosome-like structures in plasma from patients with SLE (McCoubrey et al., 1984; Li and Steinman, 1989; Rumore and Steinman, 1990; Burlingame et al., 1993). The formation of anti-nucleosome antibodies and nucleosome-Ig complexes is a characteristic feature of MRL/lpr mice. (Licht et al., 2001). Attempts to immunize non-autoimmune mice with nucleosomes prepared in vitro have failed, giving support to the notion that qualitative modifications of nucleosomes are necessary for autoantibody
induction (Amoura et al., 1999). It has been shown that during apoptosis, a series of post-translational protein modifications, including proteolysis, phosphorylation, oxidation with heavy metal etc, may create modified autoantigens that might contribute to the bypass of tolerance that is required for autoantibody formation (Utz and Anderson, 1998). An inflammatory milieu as well as release of oxygen species (Casciola et al., 1994), by activated phagocytes may affect the immunogenicity of the autoantigens. In this regard, DNA damaged by reactive oxygen species in vitro becomes immunogenic and triggers an anti-dsDNA antibody response upon injection into rabbits (Cooke et al., 1997).

It has been reported from our laboratory that immunization of reactive oxygen species modified DNA induces antibodies, exhibits polyspecificity (Ara and Ali, 1992; 1993; Alam et al., 1993) and recognizes B-, A- and allied conformations of DNA (Ara and Ali, 1995). Monoclonal antibodies against ROS-DNA have been used as an immunochemical probe to detect oxidative DNA lesions in cancer, ageing and SLE (Ashok et al., 1997; Ahmad et al., 1998).

**Free radical biochemistry**

The role of free radicals in health and disease has been widely accepted into the biochemical and medical orthodoxy. A free radical is a chemical species, possessing a very short half-life that contains one or more unpaired electrons. Free radicals are generally very reactive. They can be positively or negatively charged or electrically neutral. Free radicals can be formed by homolytic fission of a covalent bond, or by the loss/addition of a single electron from/to a normal molecule. The electron transfer is a more common process in biological systems than homolytic fission. It is now well established that free radicals and other reactive oxygen species (ROS) are continuously produced in vivo, and can damage most cellular components (Martinez-Cayneda, 1995). Free radicals are generated in vivo by oxidant enzymes, phagocytic cells, redox-cycling drugs, ionising radiations etc (Halliwell and Gutteridge, 1989). These radicals cause damage to a number of macromolecules including lipids and proteins (Lunec et al., 1985; Wolff et al., 1986; Lunec, 1990). In consequence, several antioxidant defence systems limit their damaging effects and the repair systems prevent the accumulation of

The term reactive oxygen species (ROS) includes oxygen-centered radicals as superoxide ($O_2^-$) and hydroxyl radical (·OH), and also some non-radical derivatives of oxygen such as hydrogen peroxide ($H_2O_2$), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl) and ozone ($O_3$), that are involved in oxygen radical production. ROS are highly reactive and have extremely short half-lives. There are numerous mechanisms for generation of ROS in vivo (Simic et al., 1988; Emerit et al., 1990). It has been proposed that many of the damaging effects could be attributed to chemically reactive species like superoxide, hydrogen peroxide and hydroxyl radical (Halliwell, 1987; Halliwell and Gutteridge, 1989).

Hydrogen peroxide is ubiquitous in biological systems, formed by the divalent reduction of dioxygen or by dismutation of the superoxide anion radicals ($O_2^-$) catalysed by superoxide dismutase (SOD) (Tachon, 1989).

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2.$$  

Hydrogen peroxide that escapes destruction can act as an oxidizing agent, but is not especially reactive. Normal cellular level of hydrogen peroxide is in the range of $10^{-8}$-$10^{-9}M$ (Oshino et al., 1973). It can be elevated as a result of inflammation where the respiratory burst of phagocytic cells occur (Hammers and Roos, 1985). Its main significance lies in it being a source of hydroxyl radicals in the presence of reduced transition metal ions via Fenton reaction (Fenton, 1894; Halliwell and Gutteridge, 1990).

$$H_2O_2 + Fe^{2+} / Cu^+ \rightarrow 'OH + OH^- + Fe^{3+} / Cu^2$$  

In a biosystem, where reducing conditions prevail, Fe$^{2+}$ / Cu$^+$ can be regenerated easily by an electron donor.

$$Fe^{3+} / Cu^{2+} + e^- \rightarrow Fe^{2+} / Cu^+.$$  

Hydroxyl radical can also be formed by the interaction of superoxide anion with $H_2O_2$ through Haber-Weiss reaction (Haber and Weiss, 1934).

$$O_2^- + H_2O_2 \rightarrow O_2 + H_2O + 'OH$$
Beauchamp and Fridovich in 1970 proposed that toxicity of superoxide radical and hydrogen peroxide could involve their conversion into a much more reactive hydroxyl radical. Nitric oxide, another reactive oxygen species, interacts with superoxide radical to generate peroxynitrite, which in turn forms hydroxyl radicals (Saran et al., 1990; Inoue and Kawanishi, 1995).

The hydroxyl radical formed in vivo is an extremely reactive oxidizing radical that reacts with most biomolecules at diffusion-controlled rates. It has an extremely short half-life. It does not diffuse a significant distance within a cell and is capable of causing great damage within a limited radius at its site of production.

Superoxide anion radical (O$_2^-$) is formed in all aerobic organisms. Systems generating O$_2^-$ have been observed to kill bacteria, inactivate viruses, damage enzymes and membrane and destroy animal cell culture (Fridovich, 1978; Halliwell, 1981). It is formed by one electron reduction of oxygen (Florence, 1990; Harris, 1992). Sources of superoxide in injured tissues include xanthine oxidase, mitochondria, neutrophils catecholamines. Xanthine oxidase is an important source of oxygen derived free radicals in reperfused tissue (Granger et al., 1981; Chambers et al., 1985; McCord et al., 1985; Hearse et al., 1986). Production of superoxide by mitochondria has been known for nearly two decades (Boveris et al., 1976; Turrens and Boveris, 1980). The rate of superoxide production by mitochondria increases when the concentration of oxygen is increased or the respiratory chain becomes largely reduced (Turrens et al., 1982).

A potentially large and significant source of free radicals in stimulated neutrophils is the activated NADPH oxidase of phagocytic cells. After initiation of the respiratory burst, more than 90% of the consumed oxygen can be accounted for the generation of superoxide. Production of O$_2^-$ and H$_2$O$_2$ by neutrophils is enhanced after the cells adhere to surfaces or after they are primed with a chemical stimulus (Dahinder et al., 1983).

The superoxide radical is generated within aerobic biological systems during both enzymatic and non-enzymatic oxidation. It is eliminated by conversion to H$_2$O$_2$ and O$_2$ by superoxide dismutase (Fridovich, 1983; 1986). The finding that O$_2^-$ is produced by some enzymes and is efficiently scavenged by others (McCord and Fridovich, 1968; 1969) led to the view that O$_2^-$ is an agent of oxygen toxicity. In this view the superoxide
dismutases (SODs), which catalytically scavenge O$_2^\cdot$-, serve a defensive role (McCord et al., 1971). O$_2^\cdot$- is produced in aerobic living cells and it constitutes a threat to these cells and the SODs provide necessary defense. Thus, SOD is abundant in aerobes and is scarce, or lacking entirely, in sensitive obligate anaerobes (McCord et al., 1971).

Singlet oxygen (¹O₂) is a reactive oxygen species (ROS) involved in a variety of biological functions such as gene expression, photoaging and apoptosis (Grether Beck et al., 1996; Ryter and Tyrrell, 1998; Zhuang et al., 1999; Krutmann, 2000). It was discovered by Kantsky and deBruijn in 1931. ¹O₂ is a chemically aggressive oxygen species, capable of attacking cellular components critical for cell survival. Ever since its discovery, its production by photosensitization reactions (including those involving endogenous sensitizers) has been intensively investigated but more recently attention has been drawn by studies showing that ¹O₂ can also be generated in the absence of light, e.g., by lipid peroxidation, by a number of enzymatic reactions (Gille and Joenje, 1991) or by interaction between superoxide and reduced glutathione (Wefers and Sies, 1983). Singlet oxygen is also produced during photo-oxidation of a variety of biological compounds and xenobiotics (Krinsky, 1977; Krasnovsky, 1991). It has been established that human leukocytes can generate ¹O₂ (Kanofsky et al., 1988). Singlet oxygen is relatively long-lived, with half- times in the range of 4-50 μs, so that diffusion of singlet oxygen is possible within a radius estimated to be in the range of 100 Å (Schnuriger and Bourdon, 1968; Moan, 1990). Reactions of ¹O₂ are physical and/or chemical (Kasha and Chan, 1970; Krasnovsky, 1979). The physical reactivity is characterized by photoemissive decay. The chemical reactions are manifold, including, addition to olefins, forming dioxetanes, allylic hydroperoxides (ene reaction), and endoperoxides, as well as oxidation of sulfides or phenols to form sulfoxides or hydroperoxydienones (Wasserman and Murray, 1979; Frimer, 1985; Aubry, 1991). This chemical reactivity is the basis of biological damage inflicted by singlet oxygen.

**Free radical production in cells**

Production of free radicals in animal cells can either be accidental or deliberate. The major source of free radicals in cells is electron ‘leakage’ from electron transport...
chains, such as those in mitochondria and in the endoplasmic reticulum to molecular oxygen, generating superoxide. Auto-oxidation of certain compounds including ascorbic acid (vitamin C), thiols (e.g. glutathione, cysteine), adrenaline and flavin co-enzymes is also a good source of superoxide. Activated phagocytes also deliberately generate superoxide as part of their bactericidal role (Babior, 1978). Several toxic foreign compounds enhance the free radical production in cells. Flavin oxidases located in the peroxisomes also produce superoxide or hydrogen peroxide. The generation of reactive free radicals overwhelms the antioxidant defense in the liver and results in serious tissue damage. In many cases, the free radical production may be secondary to the initial toxic mechanism, a consequence rather than the cause of cell damage.

**Biological effects of free radicals**

The activated oxygen species, including superoxide anion radical, hydrogen peroxide, hydroxyl radical and singlet oxygen can be generated by the incomplete reduction of oxygen to water during respiration, by exposure to radiation, lights, metals, redox active drugs or by release from stimulated macrophages (Sies, 1986). These species may reach the genetic material yielding DNA damage by a variety of mechanisms (Halliwell and Aruoma, 1991).

Hydroxyl radicals resulting from oxidative metabolism or endogenous sources such as ionizing radiations or redox cycling drugs have been shown to induce base damage (Aruoma et al., 1989, 1991; Blakely et al., 1990; Gajewski et al., 1990), strand breakage (Bradley and Erickson, 1981; Kohen et al., 1986), DNA-protein cross links (Mee and Adelstein, 1979, 1981; Lesko, 1982; Chiu et al., 1986, 1993; Gajewski et al., 1988; Nackerdien et al., 1991), and other types of DNA damage (Hutchinson, 1985 and von Sonntag, 1987). Hydroxyl radical attacks all constituents of DNA producing a multiplicity of chemical changes in the deoxyribose, pyrimidines and purines (von Sonntag, 1987). Hydroxyl radicals are also believed to be responsible for a major part of the chromatin damage (Brawn and Fridovich, 1981; Halliwell, 1987; NassiCalo et al., 1989; Friedberg et al., 1995).
The damaging effects of hydroxyl radicals in cells depend on their proximity to DNA. In most cases, however, the reaction of hydroxyl radicals with chromatin can be verified only indirectly, i.e. through the damage hydroxyl radical produces on chromosomal proteins and DNA. Direct detection of hydroxyl radicals in the presence of DNA has been very difficult to achieve, as these radicals are very short lived and highly reactive towards biomolecules (Pryor, 1988). Nackerdien et al in 1991 demonstrated the increase in production of DNA base products in isolated human chromatin such as cytosine glycol, formamidopyrimidines and 8 hydroxypurines arising from reactions of hydroxyl radical with the DNA bases.

The superoxide anion radical is formed in almost all aerobic cells. Numerous studies of the effects of O$_2^-$ flux upon erythrocytes have been reported. 1, 4-napthoquinone-2-sulfonate reacts with oxyhemoglobin yielding methemoglobin plus O$_2^-$ and can be used to increase O$_2^-$ production in erythrocytes (Goldberg and Stern, 1976). Dismutation of O$_2^-$ yields hydrogen peroxide (Halliwell and Gutteridge, 1989). The latter compound can cause formation of modified bases from pyrimidines and purines in the presence of metal ions in isolated DNA (Aruoma et al., 1989, 1991; Blakely et al., 1990), in isolated mammalian chromatin (Dizdaroglu et al., 1991 b; Nackerdien et al., 1991), and in intact mammalian cells (Dizdaroglu et al., 1991 a). Several studies have implicated both O$_2^-$ and H$_2$O$_2$ in the lung damage caused by hyperoxia.

Singlet molecular oxygen (¹O$_2$) has also been implicated in several biological processes that may lead to genetic damage. DNA is one of the main targets of ¹O$_2$, it has been demonstrated that ¹O$_2$ reacts preferentially with guanine residues either as free nucleosides (Cadet et al., 1983) or as components of the DNA molecule (Menck et al., 1993), yielding a variety of DNA lesions selectively at guanine sites. These include DNA cleavage (Devasagayam, 1991), alkali and piperidine-labile sites including abasic sites (Blazek et al., 1989), cyanuric acid (Cadet et al., 1983), 2,6-diamino-4-oxo-5-formamidopyrimidine (FapyG) and 7,8-dihydro-8-oxodeoxyguanine (8-oxodG) (Floyd et al., 1989). Studies have demonstrated that ¹O$_2$ also induces single-strand breaks in DNA (Blazek et al., 1989). The harmful effects of ¹O$_2$ are not limited to nucleic acids. Its
reactivity with amino acids as well as with lipids, leading to damage to cell membranes, is also well documented (Frimer, 1985). Singlet oxygen inhibits platelet aggregation. Studies (Stief et al., 2001) have demonstrated that $^1$O$_2$ inhibits and reverses platelet aggregation.

There are several lines of evidence supporting the idea that singlet oxygen is a major cytotoxic species towards eukaryotic cells (Eisenberg et al., 1984; Dubbelman et al., 1988), bacteria (Epe et al., 1989) and viruses (Houba-Herin et al., 1982). It has been suggested that damages caused in the nucleus by singlet oxygen could be, to some extent, responsible for cell inactivation (Ito, 1974; Kobayashi and Ito, 1976). Some evidence showing that $^1$O$_2$ can promote mutagenic effects has been provided for a variety of organisms ranging from yeast (Ito and Kobayashi, 1977) and bacteria (Gutter et al., 1977) to viruses (Piette et al., 1978). Decuyper-Debergh et al (1987) demonstrated that Guanine oxidation products induced by singlet oxygen constitute premutational lesions if they are not repaired.

A large number of other modifications of bases and sugars have been identified (Dizdaroglu, 1991; 1994). The yield of the individual DNA modifications is highly dependent on which reactive oxygen species are involved. Thus, whereas singlet oxygen induces preferentially 8-oxo-dG (Epe, 1991), superoxide has low reactivity (Fischer-Nielsen et al., 1994), and hydroxyl radical can cause almost any modification (Dizdaroglu, 1991).

**Antioxidants**

Aerobic organisms have potent antioxidant defences whose role is to neutralize and minimize the potentially cytotoxic and genotoxic effects of reactive oxidants. Antioxidants are key line of defence capable of scavenging free radicals by preventing radical formation, intercepting radicals from further activity (Cotgreave et al., 1988), or participating in repair of damage caused by free radicals (Sies, 1993). Antioxidant defences may be primary or secondary. The defences that directly scavenge, H$_2$O$_2$ and $^\cdot$OH are known as primary antioxidant defence. Secondary antioxidant defences consist
of the repair mechanisms that act on biomolecules that have undergone oxidative damage.

There may be enzymatic or non-enzymatic antioxidant defences. Enzymatic antioxidant defences include superoxide dismutase (SOD), catalase, glutathione peroxidase etc. Superoxide dismutase catalyses the dismutation of \( O_2^- \) to \( H_2O_2 \) (Fridovich, 1989).

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

The concentration of SOD is high in tissues with high oxygen utilization and is inducible by raising tissue \( pO_2 \).

Catalase mediates the detoxification of \( H_2O_2 \) from the cell when it is present in high concentrations. Catalase is a heme protein that decomposes \( H_2O_2 \) to \( O_2 \) and \( H_2O \).

\[
2H_2O_2 \xrightarrow{\text{catalase}} O_2 + 2H_2O
\]

Catalase is present in the cytosol, mitochondria and other organelles, but is difficult to detect in an extracellular environment.

Selenium dependend glutathione peroxidase (GSHpx) catalyzes the reduction of \( H_2O_2 \) and organic free hydroperoxides requiring glutathione as substrate (Emster, 1987).

\[
\begin{align*}
H_2O_2 + 2GSH \xrightarrow{\text{GSHpx}} & \quad GSSG + 2H_2O \\
\text{(reduced glutathione)} & \quad \text{(oxidized glutathione)}
\end{align*}
\]

\[
\begin{align*}
ROOH + 2GSH \xrightarrow{\text{GSHpx}} & \quad GSSG + ROH + H_2O \\
\end{align*}
\]

Glutathione reductase reduces oxidized glutathione utilizing NADPH generated by various systems.

\[
GSSG + NADPH + H^+ \longrightarrow 2GSH + NADP^+
\]

Normally, most of the intracellular glutathione is in its reduced form. Increased intracellular concentrations of \( H_2O_2 \) results in a drop in GSH/GSSG ratio, which serves to detect intracellular oxidative stress (Tribble and Jone, 1990). Other enzymatic proteins such as DT-diaphorase or epoxide hydrolase are also considered to be primary antioxidant defences (Emster, 1987; Lind et al., 1990).
There is no enzymatic mechanism to directly protect against 'OH because of its extremely high reactivity and rapid consumption. The cell possesses non-enzymatic antioxidant defence mechanisms, which scavenge 'OH. These scavengers may be hydrophillic (ascorbate, urate, glutathione) or hydrophobic (α-tocopherol, β-carotene). Glutathione, vitamin C, uric acid, taurine, hypotaurine are some of the small molecules widely distributed in biological systems, which scavenge oxygen free radicals non-enzymatically. Vitamin E (α-tocopherol), the major lipid-soluble antioxidant protects against lipid peroxidation by donating a hydrogen ion to oxygen free radical. The resultant tocopheryl radical may be reduced by the ascorbic acid-GSH redox couple (Cadenas, 1989; Empey et al., 1992). α-tocopherol is key 'OH scavenger and chain breaking antioxidant in biological membranes (Niki et al., 1988). Vitamin E is important in protecting tissue from a variety of physio-pathological insults, which results in enhanced tissue reactive oxygen species generation (Chow, 1991). β-carotene, the most efficient scavenger of singlet oxygen, has a synergistic action with vitamin E (Machlin and Bendich, 1987; Bendich and Olson, 1989; Di Mascio et al., 1991).

The various defences are complementary to each other since they metabolize or scavenge different species in different cellular compartments.

**Autoimmunity**

The ability to distinguish self from non-self is a seminal feature of the immune system. Central T-cell tolerance to self-antigens is mediated either by elimination or functional inactivation of self-reactive lymphocytes (Zouali, et al., 1993). Disruption of self-nonself discrimination leads to autoimmunity (Deodhar, 1992). Autoimmunity is a state wherein the host mounts an immune response to self. There are at least four possible immune states in an autoimmune response (Brickman and Shoenfeld, 2001) (Table 1). Normally, the immune system responds to a wide variety of foreign insults, such as bacteria, viruses, other parasites and internal changes such as cancer, while not responding to one's own self antigens. During immunological disbalance, as a result of internal threat, the body's own tissue components become reactive and may result in the initiation of autoimmune process / diseases (Deodhar, 1992). There is a delicate balance
TABLE - 1

Possible immune states in an autoimmune response

1. Healthful immune state in an otherwise normal host: Anti-idiotypic antibodies modulating an acute antibody response following an acute, self-limited infection.


3. Detrimental immune state in the abnormal host: Immune response to malignant cells cross reacts with normal tissue antigens causing an autoimmune disorder.

4. Detrimental immune state in the otherwise normal host: Results in classic autoimmune, clinico-pathologic conditions such as lupus, diabetes or thyroiditis.

between autoimmunity and autoimmune disease. Autoimmunity may be envisioned a normal event, while autoimmune diseases result from an aberration of this normal phenomenon. Autoimmunity, namely the ability to recognize various forms of self, is a normal function of the immune repertoire. In contrast, autoimmune disease is the clinicopathologic state wherein the host mounts a detrimental response to self (Brickman and Shoenfeld, 2001).

The generation of autoimmunity is multifaceted process in which the role of the autoantigen needs to be carefully defined (Zouali et al., 1993). Autoimmune diseases are characterized by more or less systemic chronic inflammatory processes, possibly leading to tissue damage (Lorenz et al., 2001).

Factors associated with autoimmune disease

Autoimmune disease is multifactorial. Criteria for classification of a disease as autoimmune have been established (Rose and Bona, 1994) and these research criteria exist for the classification of most autoimmune disorders. The factors contributing to the development of such diseases continue to expand. The major factors associated with the development of autoimmunity maybe divided into genetic, immunologic, hormonal and environmental (Brickman and Shoenfeld, 2001).

(i) Genetic factors

Autoimmune diseases show a highly significant familial predisposition (Hochberg, 1987; Arnett, 1992). Clinicians treating patients with autoimmune disorders have long been struck by the finding that such patients frequently have relatives with the same or with other autoimmune disorders (Shoenfeld and Isenberg, 1989). The involvement of genetic factors has been linked to the human lymphocyte antigen (HLA) system, particularly the HLA-DR sublocus. The HLA genes function as secondary genes to allow expression of specific autoantibody or the respective disease state (Bias et al., 1986). The HLA molecules that are present on the surface of all nucleated cells and platelets are encoded for within the major histocompatibility complex (MHC) on the short arm of chromosome 6 in humans (Burnett, 1959). The initiation of an autoimmune
response requires that the self-reactive T cells interact with self-antigen and HLA class II antigen complex with sufficient avidity for the development of autoantibody in subsequent autoimmune diseases. The association between certain autoimmune diseases and HLA antigens (Braun and Zachary, 1988) such as SLE DR2 and DR3, Sjogren's syndrome and DR2 and for DR3, insulin-dependent diabetes mellitus or diabetes Type I DR4 and DR3, Grave's disease and DR3, myasthenia gravis and DR3, rheumatoid arthritis and DR4 are well documented. However genetics is not the final determinant of autoimmune disease development (Brickman and Shoenfeld, 2001). If a single gene were solely responsible for autoimmune diseases, homozygotic twins would show 100% concordance, not, for example, the 57% concordance reported in lupus and the 34% reported in rheumatoid arthritis (Winchester and Lahita, 1987).

(ii) **Immunologic factors**

Cellular immunity with anti-self properties is part of the normal immune response. Low affinity polyspecific antibodies and idiotypic antibodies may play a role in immunoregulation. Malregulation of immune system depends on multiple components of feedback regulation system including T cells, B cells and idiotypes (Shoenfeld and Schwartz, 1984). In a wide a variety of autoimmune diseases, the regulatory failure result in significant decrease in T-suppressor cell numbers and activity, thereby imbalancing the T-helper/T-suppressor cell ratio. The increased T-helper/T-suppressor cell ratio had been noted in a wide variety of autoimmune diseases, such as, SLE, Sjogren's syndrome, PSS or scleroderma, rheumatoid arthritis, pernicious anemia, multiple sclerosis, immune complex mediated renal diseases, immunologic skin diseases and many others. Immunologic cross reactivity and molecular mimicry has been an important phenomenon in autoimmune diseases. It has been suggested that the degree of sequence conservation between host and a given infectious agent, heat shock proteins, because of molecular mimicry, may provide the link between infection and subsequent autoimmunity.

Some persons may mount an immune response against self and others do not because HLA molecules from different individuals display different portions of the antigen to T cells. Thus, one person's HLA molecule may bind a self-mimicking portion
while another's may not. Polyclonal B cell activation has been proposed as one possible mechanism that may be responsible for the over-activation of B cells and production of autoantibodies in certain autoimmune diseases, particularly SLE (Dziarsky, 1988; Klinman et al., 1990; Steinberg, 1992). In autoimmune prone individuals, B cells are hyper-responsive to polyclonal activators and undergo initial activation, followed by expansion of auto reactive clones, under the influence of exogenous or endogenous polyclonal activators like Epstein Barr Virus (EBU) and its components and endotoxin or lipopolysaccharide (LPS), certain bacterial agents and drugs.

One hypothesis called as the 'modified self' hypothesis suggests that autoimmunity may arise as a result of an immune response against modified self determinants (neo-self determinants) such as new determinants created on somatically mutated antibodies during the maturation of the immune response. Rheumatoid factors (RFs), which are anti-IgG Fc autoantibodies induced transiently during an immune response, presumably because neo-antigenic determinants are exposed on antigen complexed IgG. Glycosylation defects in IgGs have also been proposed as playing a role in RF induction, although the specificity of this phenomenon is uncertain (Tsuchiya et al., 1993). Complement deficiencies (Fries et al., 1986) associated with particular HLA phenotypes may cause immune disease via inefficient opsonization of infectious agents or slowed clearance of immune complexes (Frank et al., 1983). "Nephritic factor" referring to an antibody against neo-antigenic determinants reveals the complement protein C3 during complement activation (Spitzer et al., 1992). Other possibilities of creating non-self determinants occur through building of drugs or other haptenic groups to self-molecules, as well as through molecular modifications introduced, for example, by gene mutations.

(iii) **Hormonal factors.**

Hormones are a significant factor in the development of autoimmune disorders (Ahmed and Talal, 1999; Lahita, 1999). Clinicians caring for patients suffering from these disorders were the first to bring to the scientific community's attention the predilection of some (e.g. rheumatoid arthritis and lupus), but not all, autoimmune
diseases for females. Patients born with Klinefelter's syndrome, a feminising genotype, also have an unusually high prevalence of lupus (Ortiz and LeRoy, 1969). Animal models of autoimmune diseases show similar predilections. Despite this, the role sex hormones play in autoimmune disease is unclear. In humans as well as in animal models, some autoimmune diseases show a predilection for males rather than for females. Danazol, a semi-synthetic androgen occasionally used in treatment of autoimmune disorders, may induce lupus in some patients (Guillet et al., 1988). Other hormones including progesterone (VanVollenhoven and McGuire, 1994) and prolactin (Walker et al., 1998) appear to have immunoregulatory properties.

(iv) Environmental factors

Environmental factors have been implicated in autoimmune diseases including infectious agents, medications, chemicals, toxins and ultraviolet light to name but a few (Aharon and Shoenfeld, 1998; Saraux et al., 1999). Smoking and obesity may increase the risk of RA (Karlson et al., 1999; Shovman and Shoenfeld, 2000). Here, the multifactorial nature of autoimmune disorders also become evident while ultraviolet light is known to trigger lupus (McGrath, 1999), it has been very effectively used to treat psoriasis, another autoimmune disease (Halpern et al., 2000).

D-pencillanmine, a medication formerly used to treat several immune diseases including RA, scleroderma and primary biliary cirrhosis, has been implicated in the development of autoimmune diseases such as lupus and glomerulonephritis (Brik et al., 1995). Physical and psychological stresses have also been implicated in the development of autoimmune diseases (Gaillard and Spinedi, 1998).

The common thread among all the hypotheses of autoimmune diseases is the loss of energy to innocent autoantigens or the loss of tolerance to self. However, to date no single theory adequately explains the development of all autoimmune diseases and, at least in humans, no one theory adequately explains the development of particular autoimmune disorder. Therefore, it may be stated that the evolution from autoimmunity to autoimmune disease is multifactorial (Brickman and Shoenfeld, 2001).
Autoimmune diseases may be classified, somewhat arbitrarily, into organ specific and systemic autoimmune diseases (Table 2). There are autoimmune diseases in which the autoantigen is known, like myasthenia gravis (acetylcholine receptor), Grave's disease (TSH-receptor), or encephalomyelitis disseminata (myelin basic protein).

**Systemic lupus erythematosus**

Systemic lupus erythematosus (SLE) is a systemic autoimmune disorder characterized by a marked diversity of organ involvement and fluctuations in disease activity (Min *et al.*, 2002). It is a relatively common non organ-specific disease. Characteristically, inflammatory skin lesions and multiple organ damage occur. In 60-70% of cases, renal involvement complicates the systems and its severity largely determines prognosis (Zouali, 2001). Since these systems are associated with autoantibody production against a myriad of nuclear antigens, SLE has become a prototype of systemic autoimmune diseases (Hahn, 1998; Davidson and Diamond, 2001). With a prevalence comparable to that of multiple sclerosis, it predominantly affects women, with a female/male ratio of approximately 9/1 (Zouali, 2001). SLE is the most representative of all autoimmune diseases because it may affect any organ of the body and display a wide range of clinical manifestations (Cervera *et al.*, 1993) and various immunologic disorders, including production of autoantibodies, formation of immune complexes, decreased serum complement levels, and lymphocytopenia (Kato *et al.*, 2000).

SLE is characterized by the production of a variety of autoantibodies against nuclear, cytoplasmic and cell surface antigens. The cellular and molecular mechanisms that are responsible for the production of antinuclear antibodies in this disease and the way in which these antibodies participate in tissue destruction remain highly controversial (Ravirajan *et al.*, 2001).

One of the first autoantibody populations to be characterized was antibodies to native DNA, which are strongly correlated with the diagnosis of SLE (Tan, 1989). In 1997, the discovery of autoantibodies reactive with DNA celebrated its 40th anniversary. Over these four decades, perhaps no other single spontaneously produced autoantibody
<table>
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<th>Organ specific autoimmune diseases</th>
<th>Systemic / non-organ specific autoimmune diseases</th>
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<td>Grave’s diseases</td>
<td>Systemic lupus erythematosus</td>
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<td>Hashimoto’s thyroiditis</td>
<td>Sjogren’s syndrome</td>
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<td>Rheumatoid arthritis</td>
<td>Systemic sclerosis</td>
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<td>Myasthenia gravis</td>
<td>Mixed connective tissue damage</td>
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<td>Pernicious anemia</td>
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<td>Addison’s disease</td>
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<td>Insulin dependent diabetes mellitus</td>
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<td>Multiple sclerosis</td>
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<td>Systemic lupus erythematosus</td>
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<td>Sjogren’s syndrome</td>
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<td>Mixed connective tissue damage</td>
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has drawn such a wide scientific interest from basic immunologists and clinicians, particularly with respect to the mechanisms that lead to anti-DNA antibody formation and pathogenicity in systemic lupus erythematosus (Antonio et al., 1998). Anti-dsDNA antibodies are a hallmark of SLE (Tan et al., 1966; Arana and Seligmann, 1967), and anti-dsDNA/DNA immune complexes have long been considered responsible for the development of lupus nephritis. The presence of anti-dsDNA antibodies in the serum of SLE patients constitutes one of the eleven American Rheumatism Association (ARA) criteria for the diagnosis of SLE (Tan et al., 1982). It defines a clinical subset of the disease and provides information of the prognosis and response to treatment (Pisetsky, 1992). As structural studies with a growing number of monoclonal anti-DNA antibodies have progressed, it has emerged that anti-dsDNA antibodies have all the characteristics of those produced in an antigen-stimulated secondary immune response. They are predominantly of IgG isotype, are highly oligoclonal, and have numerous somatic mutations in their \( V_H \) regions, features typical of an antigen driven response (Eilat and Fischel, 1991; Tillman et al., 1992; Radic and Weigert, 1994).

Autoantibodies to histones were found to be present as often as anti-DNA antibodies in SLE (Gioud et al., 1982; Hardin and Thomas, 1983; Monestier and Kotzin, 1992). Several studies have demonstrated that anti-native DNA antibodies are commonly copresent with anti-histone antibodies in SLE sera (Costa and Monier, 1983; Krippner et al., 1984; Kohda et al., 1989). Anti-histone antibodies may react with each of the five histones (Hardin and Thomas, 1983; Portanova et al., 1987; Muller et al., 1989; Monestier and Kotzin, 1992) and with H3-H4 and H2A-H2B complexes (Burlingame et al., 1994). The importance of anti-histone antibodies in SLE is confounded by discrepancies in their reported prevalence, isotype, specificity and correlation with symptoms (Monestier and Kotzin, 1992; Rubin, 1992). As a result, little insight into their diagnostic ability, pathogenic significance or origin in SLE has been derived (Suzuki et al., 1994).

The concomitant presence of autoantibodies to histones and to DNA in the same individual led to the early hypothesis that these two populations were linked sets of
antibody and were induced by a unique antigen composed of both dsDNA and histones (Hardin, 1986).

Serum antibodies reacting with chromatin or its predominant components, histones and DNA, have been found in SLE, drug induced lupus, several other human disease states and in murine models of SLE (Schwartz and Stollar, 1985; Theofilopoulos and Dixon, 1985; Costa and Monier, 1986; Fisher et al., 1988; Portanova et al., 1988; Burlingame and Rubin, 1991). Several studies have demonstrated that the autoimmune response is against chromatin with the development of antibodies directed to various epitopes on chromatin including but not limited to DNA (Burlingame et al., 1993; 1994; Mohan et al., 1993; Amoura et al., 1994; Chabre et al., 1995; Tax et al., 1995). A central role for chromatin in autoimmune responses to histones and dsDNA in human lupus has been suggested (Burlingame et al., 1994). It has been suggested that the nucleosome, the fundamental repeating unit of chromatin, could be a major autoantigen in lupus. Circulating nucleosome oligomers have been demonstrated in the plasma of SLE patients. (Rumore and Steinman, 1990). Studies have revealed that anti-nucleosome antibodies occur early in life, before the emergence of anti-dsDNA and anti-histone antibodies (Burlingame et al., 1993; Amoura et al., 1994).

The nucleosome (or chromatin) is emerging as the most reactive substrate among the nuclear antigens, 70-80% of SLE patients being positive (Burlingame et al., 1994; Wallace et al., 1994; Chabre et al., 1995; Lefkowith et al., 1996; Amoura et al., 1997). Anti-nucleosome antibodies might be a good marker of anti-DNA negative SLE cases (Amoura et al., 1999; Min et al., 2002).

Other autoantibodies that have been demonstrated in sera of SLE patients include anti-ribosomal P protein antibodies, anti-Ro/La autoantibodies, anti-neutrophil cytoplasmic antibodies, anti-phospholipid/cofactor antibodies, antinuclear antibodies (reacting with ssDNA, dsDNA, ribonucleoprotein, Sm etc) (Anasuma et al., 1997; Emlen and O’Neill, 1997; Font et al., 1997; Guerin et al., 1997; Lang et al., 1997; Villarreal et al., 1997; Wang et al., 1997; Anderson et al., 1998).

Anti-DNA antibodies are deposited in kidneys of patients and mice with lupus diseases. In lupus, nucleosomes could be at work in the induction of anti-DNA antibodies
(Mohan et al., 1993), and in the development of kidney lesions (Tax et al., 1995). Observations suggest that cationic residues of histones could bind to anionic heparan sulphate and mediate the glomerular deposition of autoantibodies. Deposition of histones has been found in glomeruli from mice and humans with lupus (Schmiedeke et al., 1992). The mechanisms by which the nucleosome-triggered deposition of pathogenic antibodies is mediated (immune complex deposition or/and local formation) is still a matter of debate (Fournie, 1996). The issue of whether lupus autoantibodies are the result of antigen stimulation or of polyclonal activation, has been addressed by a number of investigators. B-lymphocytes from SLE patients generally appear to be more activated than B cells from healthy individuals. Further molecular studies of autoantibody genes revealed that in both the human disease and in experimental models of lupus, production of autoantibodies is antigen driven (Shlomchik et al., 1990; Manheimer-Lory et al., 1991; Winkler et al., 1992; Zouali, 1992; 1997).

Autoantibodies and B cells have been in the lupus limelight for decades, but now T cells have been studied extensively in SLE (Spronk et al., 1996). T cells have been cloned from lupus-prone mice, and stimulate the production of anti-DNA antibodies and renal lesions when injected in vivo. In mice and humans with lupus, the histone/DNA binding T cells are activated, express the CD40 ligand, and have the potential to trigger B cells to produce antibodies. Various T cell abnormalities have been observed in SLE (Tsokos, 1992; Horwitz and Stohl, 1993; Via and Handwerger, 1993; Dayal and Kammer, 1996; Datta et al., 1997a; Horwitz, 1997; Kovacs, 1997). The T cells of patient with SLE show impaired in vitro proliferation in response to mitogens, antigens and allogenic major histocompatibility complex (MHC) molecules (Via and Handwerger, 1993; Dayal and Kammer, 1996; Datta et al., 1997a; Horwitz, 1997).

The complement system is of central importance in SLE. Acquired complement deficiency is a common finding in SLE. Low C3 and C4 complement suggests, that there is activation of the classic complement pathway in active immune complex disease. Measurement of classic pathway complement components is important in the diagnosis of SLE and for monitoring of immune complex mediated manifestations especially proliferative glomerulonephritis (Sturfelt, 2002).
Oxygen free radicals in SLE

Oxygen derived species such as superoxide ($O_2^-$) and $H_2O_2$ are produced in mammalian cells as a result of aerobic metabolism (Fridovich, 1978; Halliwell and Gutteridge, 1985). Excess generation of these species can result in damage to macromolecules, including DNA (Aruoma et al., 1989b) and have been implicated in etiology of many human diseases (Halliwell and Gutteridge, 1990; Lunec, 1990), including SLE (Blount et al., 1994; Cooke et al., 1997). DNA damage by reactive oxygen species (ROS) has been found in the development of autoimmune diseases, such as SLE (Blount et al., 1991). The process may involve the release of ROS intermediates from activated phagocytic cells, their passage through cell membranes, and finally reaction with nuclear DNA (Bashir et al., 1993) producing altered DNA, stimulating DNA-antibody production. The development of autoantibodies in SLE has been supported by the enhanced reactivity of SLE anti-DNA antibodies to ROS modified DNA and polynucleotides (Blount et al., 1989; 1990; Alam et al., 1993; Ara and Ali, 1993; Ahmad et al., 1997; Cooke et al., 1997). The detection of 8-oxo-dG in the immune complex derived DNA of SLE (Lunec et al., 1994), reinforces the evidence that ROS may be involved in SLE. ROS modified DNA may play a significant role in the generation of immune complexes, which are of recognized importance in the pathogenesis of SLE (Lisitsyna et al., 1996; Cooke et al., 1997).

Approach to therapy

Despite the power of modern molecular approaches and persisting investigative efforts, lupus remains an enigmatic disorder. Corticosteroids and cyclophosphamides are widely used for the treatment of SLE, which exert immunosuppressive activities (Kroemer and Martinez, 1994). However, their utility is restricted by their undesirable side effects including infection, premature cardiovascular mortality, infertility, and neoplasia (Hellmann et al., 1987; Pistiner, 1991). Immunosuppressive drugs with a high desired/adverse reaction ratio are awaited. It has been shown that FTY720 [2-amino-2-($z$-{4-octyl-phenyl}ethyl)-1,3-propane-diol hydrochloride], a novel immunosuppressant is
efficacious for the treatment of experimental SLE in MRL/lpr mice (Okazaki et al., 2002).

If lupus is due to a loss of self-tolerance, it may be possible to design a more
general therapy to restore self tolerance (Steinberg, 1994). Gene therapy, another
approach to curing of lupus has been performed successfully in mice. Provided that
kidneys are not yet irreversibly damaged, the use of human soluble or chimeric receptors
(constant part of human IgG heavy chain linked to the human soluble IFN-receptor)
(Kurschner et al., 1992 a, b) is an attractive prospective in the treatment of autoimmune
glomerulonephritis in human SLE (Ozmen et al., 1995). Specific immunotherapy for
human SLE can be designed based on the studies of Datta et al., 1997b. Once the
pathogenic Th cell epitopes in the nucleosomes are identified, autoantigen mediated
signal 1 to the Th cells could be blocked, in combination with anti-CD40-L therapy to
block CD40-mediated signal 2 to the pathogenic autoantibody producing B cells. Thus
instead of blocking signals 1 and 2 for either the autoimmune T or the autoimmune B
cell, both of these major players in the pathogenic unit could be blocked by combination
therapy.

Cancer

Carcinogenesis is the malignant transformation of a cell or group of cells (Farber
and Cameron, 1980; Potter, 1983; Farber, 1984). Almost every tissue in the body can
spawn malignancies, some even yield several types, with each cancer having unique
features. The genes implicated in the malignancy are often modified forms of the human
genes. The activation of proto-oncogenes into oncogenes, the product of which when
altered contribute to malignancy. Mutation can also convert proto-oncogene into
carcinogenic oncogenes.

As with other chronic diseases, cancer too has a multifactorial etiology which
include both genetic and environmental factors (Gourley et al., 1992). Genetic influence,
though long been suspected in incidence of cancer, is less conspicuous and more difficult
to identify (Clemens, 1991). The environmental factors are responsible for maximum
percentage of all human cancers.
Among the environmental factors alcohol consumption is associated with cancer of oesophagus, liver and rectum (Kabat et al., 1986; Eskelson et al., 1993). Tobacco consumption is the major cause of cancer of lung, larynx, mouth, pharynx, bladder, pancreas and probably Kidney (WHO, 1983). Dietary factors such as food additives, contaminants, high fat diet have been, related to cancer (Ames, 1983) Viruses such or Hepatitis B and C are related to hepatocellular carcinoma (Blumberg et al., 1975). Occupational exposures to benzene, arsenic, cadmium, chromium etc have also been known to cause cancer (Kasai and Nishimura, 1984; Frenkel, 1992; Kolachna et al., 1993; Lagorio et al., 1994). Other environmental factors such as sunlight, radiations, pesticides and medications are also known to be related to cancer (Ananthaswamy and Pierceall, 1990; Liehr, 1997).

Most of the exogenous carcinogens act via production of reactive oxygen species (Frenkel, 1992; Leanderson, 1993; Feig et al., 1994; Erhardt et al., 1997)

**Oxygen free radicals in cancer**

Oxygen derived species such as superoxide anion radical, hydrogen peroxide, singlet oxygen and hydroxyl radical are well known to be cytotoxic and have been implicated in the etiology of a number of human diseases including cancer (Cerutti, 1985; Halliwell and Gutteridge, 1989).

An increased production of oxygen derived species with in cells frequently leads to DNA damage by a variety of mechanisms and such species can probably initiate and promote cancer (Cerutti, 1985; Halliwell and Aruoma, 1991). The superoxide radical is formed in almost all aerobic cells (Fridovich, 1986). Any living system producing super oxide is expected to produce H$_2$O$_2$ by chemical or enzymatic dismutation of O$_2^-$. Endogenously generated oxygen derived species may cause damage to biological molecules, including DNA, by a variety of mechanisms (Halliwell and Arouma, 1991). Much of the toxicity of O$_2^-$ and H$_2$O$_2$ is thought to result from their metal ion-catalyzed conversion into highly reactive 'OH (Halliwell and Gutteridge, 1989; Halliwell and Arouma; 1991). The hydroxyl radical produces a unique and extensive pattern of chemical modifications in DNA and nucleoprotein, including modified bases and DNA
protein crosslinks (Oleinick et al., 1987; von Sonntag, 1987; Dizdaroglu, 1991; Halliwell and Aruoma, 1991). Such DNA lesions may be promutagenic and may play a role in carcinogenesis (Halliwell and Gutteridge, 1989; Halliwell and Aruoma, 1991; Breimer, 1990; Floyd, 1990). Evidence exist that DNA damage by endogenous free radicals occurs and accumulates in vivo, and that there is a steady state level of free radical modified bases in cellular DNA (Cathcart et al., 1984; Kasai et al., 1986; Adelman et al., 1988; Richter et al., 1988; Stillwell et al., 1989; Fraga et al., 1990; Fraga et al., 1991). Continuous endogenous damage to cellular DNA by free radicals and accumulation of such damage has been suggested to significantly contribute to carcinogenesis in humans (Totter, 1980; Ames, 1983; Ames, 1989). Because of their ability to damage DNA, free radicals are thought to be involved in all stages of carcinogenesis (Cerutti, 1985; Halliwell and Aruoma, 1991; Floyd, 1990). Evidence exists that tumor cells have abnormal levels and activities of antioxidant enzymes, such as superoxide dismutase or catalase leading to accumulation of \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) that induce damage to DNA (Olinski et al, 1992).

Understanding the role of free radicals at the molecular level may lead to an understanding of cancer related to free radicals. Olinski et al in 1992, investigated endogenous levels of typical free radical modified pyrimidines and purines of DNA in chromatin sample isolated from various human cancerous tissues and their cancer-free surrounding tissues. In all tissue types examined, the endogenous amounts of most pyrimidine- and purine-derived DNA lesions in cancerous tissues were found to be consistently higher than in their respective cancer-free surrounding tissues. It is known that \( \text{H}_2\text{O}_2 \) treatment of mammalian cells causes formation of DNA lesions in their chromatin, most likely via site-specific \( \cdot \text{OH} \) production (Dizdaroglu et al., 1991). Epidemiological studies involving measurement of typical free radical modified DNA bases in a large variety of individual tumor tissues and their respective normal tissues may provide insight into mechanisms of carcinogenesis related to oxygen-derived species.
Cancer: The chromatin connection

For decades chromatin was considered to be an inert structure whose only role was the compacting and confining of DNA inside the eukaryotic nucleus. However, the tremendous progress in this field over the last 10 years has dramatically elevated chromatin to a key position in the control of gene activity. Its role in mediating the transformation of a normal cell into a malignant state is particularly interesting (Ballestar and Estellar, 2002). Cancer is a process driven by the accumulation of abnormalities in gene function. While many of these changes are genetic, epigenetically mediated changes or heritable changes in gene expression are being increasingly appreciated. Two key components of heritable changes that are closely tied to one another are formation of chromatin which modulates transcription and establishing patterns of DNA methylation (Rountree et al., 2001).

The most significant aspect of the cancer-chromatin connection is the recognition that the expression of key genes required to convert a normal cell to a cancer cell relies on enzymes. By alternately acetylating or deacetylating histones in the context of ATP driven chromatin remodelling, the accessibility and transcriptional competence of a gene can be determined. Much of pathological gene silencing that occurs in cancer is a consequence of the mistargeting of these enzymes (Wolffe, 2001).

DNA methylation involvement in cancer has become one of the hottest topics in cancer research. A major breakthrough in the field with in the last 5 years has been the recognition of the key role of chromatin as a mediator between DNA methylation and transcriptional silencing of genes relevant to cancer (Ballestar and Estellar, 2002). The disruption of normal methylation patterns, with both hypomethylation and hypermethylation events occurring is a hallmark of tumorigenesis (Baylin and Herman, 2000; Robertson and Wolffe, 2000). A number of studies have shown that aberrant methylation is associated with changes in the chromatin structure, in particular, nucleosome position patterning (Hennig et al., 1995; Patel et al., 1997) and histone acetylation levels (Gilbert and Sharp, 1999; Torres et al., 2000). The understanding of the mechanism by which chromatin connects DNA methylation to gene silencing is fundamental to the design of drugs that specifically reactivate the silenced tumour suppressor genes. (Ballestar and Estellar, 2002).
Objectives of the present study

An increased production of oxygen derived species such as super oxide anion radical, singlet oxygen, hydroxyl radical, hydrogen peroxide etc within the cells frequently leads to DNA damage by a variety of mechanisms and such species have been implicated in the etiology of many human diseases including SLE and cancer. The complex and non-organ specific nature of SLE has made it difficult for researchers to unravel the genetic defects and pathogenic mechanisms underlying this disease. It is not clear whether some form of DNA, such as product of viral infection or a fragment of chromatin or chemically modified DNA serves as immunogen.

In the present study, chromatin was isolated from goat liver. It was modified with hydroxyl radical (·OH) generated by UV irradiation in presence of hydrogen peroxide and singlet oxygen-superoxide anion radical (¹O₂⁻O₂⁻) generated by illumination of riboflavin. Native and modified chromatin were characterized by UV and fluorescence spectroscopy, thermal denaturation studies and nuclease S1 digestibility.

Antigenicity of native and modified chromatin was probed by inducing antibodies in rabbits. Both the native and modified chromatin induced high titer antibodies. However 'OH-chromatin and ¹O₂⁻O₂⁻-chromatin were found to be more immunogenic in comparison to native chromatin as assessed by direct binding ELISA. The specificity of induced antibodies was evaluated by competition ELISA and gel retardation assay.

In order to assess the possible role of modified epitopes in the etiology of diseases such as SLE and cancer, sera from SLE and cancer patients were investigated for the presence of antibodies to native and modified chromatin.