INTRODUCTION
The mammalian immune system functions through complex interactions between various cells and their products. Antigen specific responses by the immune system are effected by B and T lymphocytes: T cells mediate cellular immunity (for example, graft rejection) and B cells mediate humoral immunity (antibody production). Other cell types such as macrophages and natural killer cells act non-specifically to eliminate foreign elements. Discrimination between self and non-self is an important feature of the immune system. Normally, immunologic attack is directed against agents which are foreign such as bacteria, virusus, parasites and some internal changes, while it does not respond to self antigens. Since the early studies of Ehrlich, immunologists have demonstrated the presence of autoantibodies in the sera of patients with various diseases. A hallmark of most autoimmune diseases is the presence of autoantibodies against self antigens.

Autoimmunity and Autoimmune Disorders

Grabar (1975) considered autoimmunity as a normal physiological phenomenon, in which the immunological mechanisms are not necessarily 'a defense' but a physiological system of transport of metabolic and catabolic substances, i.e., a 'cleaning up' of the organism of residual products and it is the application and extension of this mechanism which may act as a defense against foreign substances. Autoimmune diseases are those which result from immunologic reactions, humoral and cellular, directed against the individual's own tissue components (Deodhar, 1992). An autoimmune disease may be organ/tissue specific, e.g., Hashimoto's syndrome, thyrotoxicosis, pernicious anemia or non-organ/tissue specific (systemic) such as systemic lupus erythematosus (SLE) and progressive systemic sclerosis (PSS). These may destroy, mimic or enhance the target
and can range in severity from being mild to fatal (Minard, 1989). There
is no single theory that can explain all the features of autoimmune
diseases. Shoenfeld and Isenberg (1989) suggested that the wide spectrum
of autoimmune diseases as the 'mosaic' of autoimmunity with its many
factors, like genetic, hormonal, immunological and environmental leading
to diverse diseases.

Autoimmune diseases show a highly significant familial predisposition
(Arnett, 1992; Hochberg, 1987). Relatives of a patient with a given
autoimmune disease are known to be at a high risk for developing the
same disease. Also, multiple autoimmune diseases are known to occur in
the same patient. These findings suggest involvement of genetic factors
which has been linked to the human leucocyte antigen (HLA) system,
particularly the HLA-DR sublocus. The HLA genes function as secondary
genes to allow expression of specific autoantibody (Bias et al., 1986).
Polyclonal B cell activation has been proposed as a possible mechanism
that may be responsible for the over activation of B cells and production
of autoantibodies in certain autoimmune diseases, particularly SLE
(Klinman et al., 1990; Dziarski, 1988). In autoimmune prone individuals.
B cells are hyper-responsive to polyclonal activators and undergo initial
activation including expression of autoreactive clones, under the influence
of exogenous or endogenous polyclonal activators. Various polyclonal B
cell activators such as Epstein Barr virus (EBV) and its components,
endotoxin or lipopolysaccharide (LPS), certain bacterial agents and drugs
may function by bypassing T cell regulatory mechanisms and activating B
cells directly. The best example of human autoimmune disease in which
such direct polyclonal activation may play a role is autoimmune thyroiditis
(Goodman and Weigle, 1981).
# TABLE 1

Autoimmune Diseases and Their Respective Autoantibodies

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Autoantibodies directed against</th>
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<tr>
<td><strong>SLE and related diseases</strong></td>
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<tr>
<td>Systemic lupus erythematosus</td>
<td>nuclear antigens (DNA, Sm, cardiolipin)</td>
</tr>
<tr>
<td>Scleroderma (PSS)</td>
<td>nuclear antigens (Scl-1, Scl-70)</td>
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<tr>
<td>Sjogren’s syndrome</td>
<td>nuclear antigens (SS-A, SS-B)</td>
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<tr>
<td>Mixed connective tissue disease</td>
<td>extractable nuclear antigen (RNP)</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Fc portion of IgG (rheumatoid factor). nuclear antigens</td>
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<tr>
<td><strong>Other autoimmune diseases</strong></td>
<td></td>
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<tr>
<td>Autoimmune thyroid diseases</td>
<td>thyroglobulin, microsomal antigen, TSI</td>
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<tr>
<td>Addison’s disease</td>
<td>adrenal cortical cell antigen</td>
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<tr>
<td>Pernicious anemia</td>
<td>intrinsic factor, parietal cell antigen</td>
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<td>Inflammatory bowel disease</td>
<td>colonic mucosal antigen</td>
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<td>Chronic active hepatitis</td>
<td>smooth muscle antigen</td>
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<tr>
<td>Primary biliary cirrhosis</td>
<td>mitochondrial antigen</td>
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<tr>
<td>Mysthenia gravis</td>
<td>acetylcholine receptor</td>
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<tr>
<td>Pemphigus vulgaris substance</td>
<td>epidermal intercellular</td>
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<tr>
<td>Bullous pemphigoid</td>
<td>skin basement membrane</td>
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<tr>
<td>Autoimmune hemolytic anemia</td>
<td>red cell membrane antigen</td>
</tr>
<tr>
<td>Multiple sclerosis and relating de-myelinating diseases</td>
<td>myelin basic protein antigen</td>
</tr>
</tbody>
</table>

(adapted from Deodhar, 1992)
The immune response is controlled mainly by the regulatory influence of T-lymphocyte subsets, T-helper and T-suppressor cells. In most autoimmune diseases, there is a significant decrease in T-suppressor cell numbers and activity, which accounts for the increased T-helper/T-suppressor cell ratio. For example, in SLE the prototype of autoimmune diseases, there is a significant decrease in T-suppressor cells during active phases of the disease (Deodhar, 1992). The increased T-helper/T-suppressor cell ratio has been noted in a wide variety of autoimmune diseases, such as SLE, Sjogren’s syndrome, progressive systemic sclerosis, rheumatoid arthritis, pernicious anemia, multiple sclerosis, immune complex mediated renal diseases and many others.

In acquired immune deficiency syndrome (AIDS), the presence of autoantibodies in respective autoimmune diseases have been demonstrated in recent years (Calabrese, 1988). The autoantibodies associated with HIV infection have included anti-nuclear, rheumatoid factor, anti-cardiolipin, anti-platelet, anti-red blood cell and anti-lymphocyte antibodies. The mechanisms by which viruses and other infectious agents initiate autoimmune phenomena are probably complex and it may include structural similarity, increased HLA expression, polyclonal B cell activation and alteration of the antigen (Shoenfeld and Isenberg, 1989). Hormonal factors such as sex hormones, thymic hormones and corticosteroids play a significant role. It has been 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). The female relatives of patients with certain autoimmune diseases have been reported to demonstrate a significant prevalence of autoantibodies and suppressor T cell defects (Miller and Schwartz, 1982).
Tolerance is a state of immunologic non-responsiveness to a substance that would be expected to evoke an immune response. Tolerance provides an essential mechanism for the prevention of self-injury. Tolerance is accomplished through three mechanisms: thymic education, thymic deletion and peripheral tolerance. Thymus is the major site for 'self-nonself' discrimination. It is here that the T cell repertoire is determined. Some cells are programmed to die (negative selection) while others are not (positive selection) (Adorini, 1993). Errors in central or peripheral tolerance at the T or B cell level have also been suggested as probable causes for autoimmunity. It has also been suggested that thymus is the critical time keeper with aging process with respect to immune responses (Rose, 1994). As the thymic cortex atrophies, the response to self antigen rises, generating the aging paradox.

Antigenicity of DNA

Native DNA has not been found an effective immunogen in normal experimental animals but antibodies that react with B-conformation (native DNA) are found in individuals with autoimmune diseases, most commonly in systemic lupus erythematosus (SLE) (Stollar, 1989; Karounos et al., 1988; Tan, 1982). Haskowa et al. (1959) immunized rabbits with DNA of thymus or Ehrlich ascites tumor cells, no antibodies were detected by complement fixation, gel diffusion or passive hemagglutination. Wilson et al. (1965) found that 36 rabbits failed to respond to calf thymus DNA. Negative results were also obtained on the injection of purified native or denatured salmon, calf thymus, phage T4 or E. coli DNA (Levine and Stollar, 1968; Barnett and Vaughan, 1966; Plescia et al., 1964; Pasternak et al., 1960).
The specificity of anti-DNA antibodies has been analysed in order to understand the nature of epitope responsible for their production (Stollar, 1986). Anti-DNA antibodies react to a variety of determinants on both single stranded (ss) and double stranded (ds) DNA (Diamond et al., 1992; Tillman, 1992; Shlomchik et al., 1990; Marion et al., 1989). Although, the precise mechanism of anti-dsDNA antibody production remains unknown, studies point to a role of DNA antigen drive (Radic et al., 1989). Anti-dsDNA antibodies display pattern of variable (V) region gene utilization that resemble conventional responses in the content of somatic mutations (Hirose et al., 1993; O'Keefe et al., 1992; Shlomchik et al., 1987). Since these mutations are associated with enhanced binding, to DNA, it is likely that DNA is the selecting antigen in vivo. Among structural features associated with dsDNA binding, a high content of arginine residues in the third complementary determining region (CDR3) of the heavy chain appears important (Shlomchik et al., 1990 & 1987).

Recent studies have, however, shown that DNA complexes with a synthetic peptide, Fus-1 can induce an anti-dsDNA response in mice (Desai et al., 1993). Anti-DNA antibodies may also result by autoimmunization with chromatin, rather than native DNA (Theofilopoulos, 1995). Immunization of experimental animals with denatured DNA, synthetic polynucleotides like poly(dT), poly(dC), poly(A), poly(I), poly(G), double stranded RNA, left handed Z-DNA and chemically modified DNA can induce immune responses (Anderson et al., 1988; Stollar, 1986). The immunogenicity of certain dsDNA structures has been well documented in animal models. Z-DNA, a left handed helix, induces a significant antibody response in experimental animals (Madaio et al., 1984). The induced antibodies are highly specific for Z-DNA and do not
bind to right handed B-DNA (Bunyaard and Pisetsky, 1994). Antibodies reactive with left handed Z-DNA arise spontaneously in the sera of patients with SLE and rheumatoid arthritis and in auto-immune MRL/lpr mice (Pisetsky et al., 1990).

DNA from various bacterial species elicit high titer responses in normal mice when immunized as complexes with methylated bovine serum albumin (MBSA) (Gilkeson et al., 1989a & b). Studies have shown that bacterial DNA differs from mammalian DNA in its content of pyrimidine clusters, patterns of base methylation and in many of its coding sequences (Wells, 1988; Cheng et al., 1985; Szybalski et al., 1966). Together these features could create local regions in bacterial DNA that are structurally distinct and rarely present in mammalian DNA. The DNA from two bacterial species, Micrococcus lysodeiktus and Staphylococcus epidermidis, was recognised by normal human sera. This finding was in contrast to normal dogma that anti-DNA antibodies are prevalent in SLE alone (Fredriksen, 1991). The immune response to foreign DNA is analogous to that of foreign proteins in that, it does not require T cell recognition. The antibodies are of IgG2 isotype, where as lupus anti-DNA are predominantly IgG1 and IgG3 (Pisetsky, 1997; Pisetsky and Drayton, 1997; Tsujimura et al., 1997; Kay et al., 1988). This pattern of isotype expression suggests that the responses to foreign DNA are distinct from the lupus anti-DNA response, which appears to be T cell dependent (Robertson et al., 1992).

Free Radical Biochemistry

A free radical is any species capable of independent existence that contains one or more unpaired electrons. It is now established that free radicals and other reactive oxygen species are continuously produced in
vivo (Halliwell, 1993). In consequence, organisms have evolved not only antioxidant defense systems to protect against them, but also repair systems that prevent the accumulation of oxidatively-damaged molecules (Sies, 1991 & 1985; Fridovich, 1989; Halliwell and Gutteridge, 1989).

The term 'reactive oxygen species' (ROS) is a collective one that includes not only oxygen-centered radicals such as superoxide \( \text{O}_2^- \) and hydroxyl \( \cdot \text{OH} \), but also some non-radical derivatives of oxygen \( \text{O}_2 \), such as hydrogen peroxide \( \text{H}_2\text{O}_2 \), singlet oxygen \( ^1\text{O}_2 \), hypochlorous acid \( \text{HOCl} \) and ozone \( \text{O}_3 \) (Halliwell, 1993). Most molecules are non-radicals. When radicals react with non-radicals, new radicals are generated. Only when two radicals meet and join their unpaired electrons, are the radicals lost. The usual major route for metabolism of molecular oxygen involves its complete reduction to \( \text{H}_2\text{O} \) by accepting four electrons (Thornalley and Bannister, 1985; Fridovich, 1978; Freiden and Osaki, 1970). However, with one electron reduction, several free radicals and \( \text{H}_2\text{O}_2 \) can be formed (Byung, 1994).

\[
\begin{align*}
\text{O}_2 + e^- & \rightarrow \text{O}_2^- \quad \text{superoxide radical} \\
\text{O}_2 + \text{H}_2\text{O} & \rightarrow \text{HO}_2 + \text{OH}^- \quad \text{hydroperoxyl radical} \\
\text{HO}_2^- + e^- + \text{H} & \rightarrow \text{H}_2\text{O}_2 \quad \text{hydrogen peroxide} \\
\text{H}_2\text{O}_2 + e^- & \rightarrow \cdot \text{OH} + \text{OH}^- \quad \text{hydroxyl radical}
\end{align*}
\]

**Superoxide Radical**

The free radical, superoxide anion \( \text{O}_2^- \) is formed by the addition of one electron to ground state dioxygen. It is unstable in aqueous solutions, due to its spontaneous reactivity with itself producing hydrogen peroxide \( \text{H}_2\text{O}_2 \) and molecular oxygen \( \text{O}_2 \) (dismutation reaction) (Cadenas, 1989;
Halliwell and Gutteridge, 1984; Fridovich, 1983). The superoxide radical in its protonated form is known as perhydroxyl radical (HO\textsuperscript{+}), which exhibits even higher reactivity.

\[ \text{O}_2^{-} + \text{O}_2^{-} + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]

**Hydrogen Peroxide**

Hydrogen peroxide is the protonated form of peroxide ion (O\textsuperscript{2−}), formed by the reduction of two electrons of molecular oxygen. Although, H\textsubscript{2}O\textsubscript{2} by definition is not considered an oxygen free radical, it nevertheless remains the most extensively studied oxygen metabolite (Harris, 1992). The dismutation of O\textsuperscript{2−} by superoxide dismutase is a major source of H\textsubscript{2}O\textsubscript{2}.

\[ \text{SOD} \quad \text{O}_2^{-} + \text{O}_2^{-} + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]

H\textsubscript{2}O\textsubscript{2} is very harmful to cells because it may cross biological membranes and can also lead to the formation of highly reactive hydroxyl radical (OH) (Aruoma et al., 1991; Pryor and Church, 1991; Pryor, 1986; Mello-Filho et al., 1984; Jones et al., 1981).

**Hydroxyl Radical**

Hydroxyl radical is an extremely reactive chemical species which can react with any biological molecule. Both, its half life (fraction of microseconds) and radius of action (30°A) are very small. The O\textsuperscript{2−} and H\textsubscript{2}O\textsubscript{2} are less reactive oxidants than \textsuperscript{'OH} but they have a longer life time which allows them to react with molecules in locations far from the site of their production (Pryor, 1986; Halliwell and Gutteridge, 1984). The hydroxyl radical is produced in living systems by at least two mechanisms: reaction of transition metal ions with H\textsubscript{2}O\textsubscript{2} and homolytic fission of water due to its background exposure to ionizing radiation (von Sonntag, 1987). First.
hydroxyl radicals are derived from the decomposition of hydrogen peroxide via the Fenton reaction (Fenton, 1894)

\[
Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^- 
\]

and second, by the interaction of superoxide with hydrogen peroxide through the Haber-Weiss reaction (Haber and Weiss, 1934).

\[
O_2^- + H_2O_2 \rightarrow O_2 + H_2O + OH 
\]

Free Radical Production in Biological Systems

Oxygen free radicals are being continuously produced in organisms and many of them are necessary to carry out certain biological reactions (Martinez-Cayuela, 1995). In addition to exogenous sources of free radicals such as ionizing radiation, tobacco smoke, pesticides and pollutants, oxygen free radicals are also produced by intracellular systems.

The autoxidation of small soluble molecules in the cellular cytoplasm may lead to the production of oxygen free radicals by concomitant \( O_2 \) reduction. Examples include catecholamines, flavins, tetrahydroproteins, quinones and thiols (Proctor and Reynolds, 1984; Fridovich, 1978). Some cytoplasmic enzymes generate oxygen free radicals as products of their catalytic cycles e.g., xanthine oxidase and aldehyde dehydrogenase (Southern and Powis, 1988; Freeman and Grapo, 1982; Rajagopalan, 1980). Hemoglobin may be oxidized producing oxygen free radicals. However, a small quantity of oxyhemoglobin is only transformed into methemoglobin due to the action of methemoglobin reductases (Valenzuela and Videla, 1989; Clark and Cowden, 1985; Halliwell and Gutteridge, 1984; Proctor and Reynolds, 1984). Reactions catalysed by lipoxygenase and cyclooxygenase in the synthetic pathway of leukotrienes.
thromboxanes and prostaglandins involve oxygen free radical production (Blake et al., 1987; Mottley et al., 1982; Mason et al., 1980). These radicals, which deactivate the cyclooxygenase enzyme, can be an important feedback control of prostaglandin synthesis. A main source of $\text{O}_2^{-}$ is the respiratory burst of phagocytic cells when they are activated. This process is due to an enzyme, reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, located on the external surface of the plasmic membrane.

Cytochrome c oxidase from the mitochondrial electron transport system catalyzes molecular oxygen reduction to $\text{H}_2\text{O}$ by acquiring four electrons and four protons. Autoxidation of ubiquinone and NADH dehydrogenase produces superoxide radicals (Beyer, 1990; Freeman and Grapo, 1982; Turrens and Boveris, 1980). Cytochromes P450 and b5 of the microsomic electron transport systems generate oxygen free radicals. During the catalytic cycle of these cytochromes, superoxide radicals may be formed (Sevanian et al., 1990; Valenzuela and Videla, 1989; Rush and Bielski, 1985). Cytochrome reductases involved in redox reactions of cytochromes P450 and b5 can also produce superoxide radicals and $\text{H}_2\text{O}_2$ when they undergo autoxidation (Sevanian et al., 1990; Freeman and Grapo, 1982).

**DNA Damage and its Repair**

The air we breathe is a double edged sword. It is of course, fundamental for life; yet, many intracellular reactions in which it participates results in the formation of oxygen-derived free radicals. These highly reactive electrophilic chemical species can not only permanently damage cells by reacting with nucleic acids, proteins and polyunsaturated lipids (lipid peroxidation) but may also lead to cell death (Knight, 1995).
The endogenous reactions that are likely to contribute to DNA damage are oxidation, methylation, depurination and deamination (Ames, 1989). The chemistry of DNA damage by several ROS has been well characterized in vitro (Box et al., 1995; Epe, 1993; Steeken, 1989; von Sonntag, 1987). Different ROS affect DNA in different ways e.g. \( \text{O}_2 \) and \( \text{H}_2\text{O}_2 \) do not react with DNA bases at all (Halliwell and Aruoma, 1991). while \( \cdot \text{OH} \) generates a multiplicity of products from all four DNA bases (Halliwell and Aruoma, 1991). Of the five major DNA components, thymine and cytosine are most susceptible to \( \cdot \text{OH} \) damage, followed by adenine, guanine and deoxyribose moiety (Saul et al., 1987). 8-hydroxyguanine (8-OH-G), is considered to be an excellent marker of oxidative DNA damage (Tritscher et al., 1996; Lunec et al., 1994; Shibutani et al., 1991), due to its high mutagenic potential (Musarrat and Wani, 1994; Guyton and Kensler, 1993; Kasai and Nishimura, 1991; Ames and Gold, 1991). It has been shown that 8-hydroxydeoxyguanine causes GC—>AT base pair substitutions (Kuchino et al., 1987), which can result in the activation of human \textit{C-Ha-ras-1} oncogene (Kamiya et al., 1992).

There is evidence to implicate oxygen free radical damage in the etiology of many chronic health problems such as emphysema, cardiovascular and inflammatory diseases, cataracts and cancer (Machlin and Bendich, 1987). Table II presents a list of diseases that involve radical reactions in mammalian systems (Knight, 1995). The data from studies on humans indicate that the important determinants of oxidative damage rate includes tobacco smoking, oxygen consumption and some inflammatory diseases, whereas diet composition, energy restriction and antioxidant supplements have a minimal influence (Brown et al., 1995; Lunec et al., 1994; Bashir et al., 1993; Tagesson, 1992).
<table>
<thead>
<tr>
<th>Table II: Diseases / Disorders Linked to Oxygen Free Radicals</th>
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<tbody>
<tr>
<td>1. Ageing</td>
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<tr>
<td>2. Atherosclerosis</td>
</tr>
<tr>
<td>a) heart disease</td>
</tr>
<tr>
<td>b) stroke</td>
</tr>
<tr>
<td>3. Brain disorders</td>
</tr>
<tr>
<td>a) hyperbasic oxygen</td>
</tr>
<tr>
<td>b) aluminium toxicity</td>
</tr>
<tr>
<td>c) neuronal lipofuscinoses</td>
</tr>
<tr>
<td>d) neurotoxins</td>
</tr>
<tr>
<td>4. Cancer</td>
</tr>
<tr>
<td>5. Cardiac myopathy</td>
</tr>
<tr>
<td>a) Keshan disease</td>
</tr>
<tr>
<td>6. Chronic granulomatous disease</td>
</tr>
<tr>
<td>7. Diabetes mellitus</td>
</tr>
<tr>
<td>8. Eye disorder</td>
</tr>
<tr>
<td>a) macular degeneration</td>
</tr>
<tr>
<td>b) cataractogenesis</td>
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<tr>
<td>9. Inflammatory disorders</td>
</tr>
<tr>
<td>10. Lung disorders</td>
</tr>
<tr>
<td>a) asbestosis</td>
</tr>
<tr>
<td>b) oxygen toxicity</td>
</tr>
<tr>
<td>c) emphysema</td>
</tr>
<tr>
<td>11. Nutritional deficiencies</td>
</tr>
<tr>
<td>12. Radiation injury</td>
</tr>
<tr>
<td>13. Repurfusion injury</td>
</tr>
<tr>
<td>14. Rheumatoid arthritis</td>
</tr>
<tr>
<td>15. Skin disorders</td>
</tr>
<tr>
<td>a) solar radiation</td>
</tr>
<tr>
<td>b) burns</td>
</tr>
<tr>
<td>c) contact dermatitis</td>
</tr>
<tr>
<td>d) Bloom syndrome</td>
</tr>
<tr>
<td>16. Toxic states</td>
</tr>
<tr>
<td>a) xenobiotics</td>
</tr>
<tr>
<td>b) metal ions</td>
</tr>
</tbody>
</table>

(adapted from Knight, 1995)
Oxidative damage to DNA has been proposed to be an important factor in carcinogenesis, a suggestion supported by experimental studies on animals and in vitro (Loft and Poulsen, 1996; Ames et al., 1995; Fraga et al., 1990; Lunec, 1990; Ames, 1989). The products of repair of these lesions are excreted in the urine and the rate of excretion of repair products in terms of oxidized bases reflect the average rate of oxidative DNA damage in the body (Loft and Poulsen, 1998). Among the possible repair products from oxidative DNA modification, 8-oxo-2' -deoxyguanosine (8-oxodG), 8-oxoguanine (8-oxoGua), thymine glycol (Tg), thymidine glycol (dTg) and 5-hydroxymethyl uracil (5-OHmU) have so far been identified in urine (Fig. 1) (Loft and Poulsen, 1996; Suzuki et al., 1995; Faure et al., 1993; Shigenaga et al., 1989; Cathcart et al., 1984). The levels of oxidized bases in lymphocyte DNA or other accessible cells will reflect the steady state levels i.e., the balance between damage and repair (Loft and Poulsen, 1998).

Oxidative DNA damage is repaired in vivo by a variety of enzymes. Strand breaks are annealed and modified bases are excised as such or as nucleotides (Demple and Harrison, 1994; Ramoter and Demple, 1993). DNA glycosylases excise bases and subsequently phosphodiester bonds on each side of the abasic site are incised by endonucleases, allowing insertion of an intact nucleotide (Loft and Poulsen, 1996). Some enzymes such as endonuclease III possess both glycosylase and endonuclease activities for repair of oxidized pyrimidines. The repair products of this excision, including thymidine and hydouracil are excreted into the urine. The formamidopyrimidine DNA glycolylase enzyme (Fpg; mutM) in E. coli repairs the oxidized purines, 8-oxoguanine and 2,6-diamino-4-hydroxy-5-formamido-pyrimidine and to a lesser extent corresponding adenine derivatives (Boiteux et al., 1992). The formamidopyrimidine-DNA
Fig. 1 Products of repair of oxidative DNA damage identified in human urine.
glycosylase protein appears to repair 8-oxodG in noncoding and actively transcribed mammalian DNA sequences with equal efficiency (Bohr et al., 1995). The Uvr ABC complex in *E. coli* repair some oxidative DNA lesions by excision of 11-13 nucleotides, including a damaged base such as Tg, 8-oxoguanine and 2,6-diamino-4-hydroxy-5-formamidopyrimidine as well as abasic sites (Czeczot et al., 1991). Oxidized nucleosides and nucleotides from the cellular pools may be incorporated into DNA and lead to AT —> GC base substitution mutations in case of 8-oxoGTP (Kamiya et al., 1992; Shibutani et al., 1991). In *E. coli* the mut M and mut T repair enzymes cooperate functionally with mut Y, an enzyme which removes adenine mis-inserted opposite 8-oxoguanine in DNA (Tajiri et al., 1995).

Double strand breaks and DNA-protein crosslinks formed by oxygen radicals are repaired either by homologous recombination or by non-homologous end joining (Henle and Linn, 1997). In homologous recombination, double strand breaks are initially processed by degrading the 5 ends to reveal 3-OH single strand overhangs (Shinohara and Ogawa, 1995). In mammalian cells, double strand breaks are predominantly repaired by non-homologous end joining and it seems that this mode of repair is mediated by V(D)J system, which rejoins blunt double strand breaks (Jackson and Jeggo, 1995).

**Biological Antioxidant Defenses**

Substances that neutralize the potential ill effects of free radicals are generally grouped in the so called antioxidant defense system (Cutler, 1984). Such a system possesses many substances which are often called as antioxidants, free radical scavengers, chain terminators or reductants (Heffner and Repine, 1989; Cutler, 1984). Aerobic organisms have potent
antioxidant defenses whose role is to neutralize and minimize the cytotoxic effects of reactive oxidants. The defenses that directly scavenge $O_2^-$, $H_2O_2$ and $\cdot OH$ are known as primary antioxidant defences. There are also secondary antioxidant defenses and consist of repair mechanisms which act on biomolecules that have undergone oxidative damage.

a) **Enzymatic antioxidant defenses**

This group includes superoxide dismutase, catalase and glutathione peroxidase.

i) **Superoxide dismutase** - The enzyme superoxide dismutase (SOD) catalyses the dismutation of $O_2^-$ to $H_2O_2$ (Fridovich, 1989).

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

SOD exists in virtually all $O_2$-respiring organisms, its activity varying among the tissues. The highest levels are seen in kidney, liver, adrenal gland and spleen.

ii) **Catalase** - Catalase is a heme protein that decomposes $H_2O_2$ to $O_2$ and $H_2O$.

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

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

iii) **Glutathione peroxidase** - The selenium based enzyme, glutathione peroxidase, reduces $H_2O_2$ by catalysing its reaction with the reduced form of glutathione (GSH).

\[ H_2O_2 + 2 \text{ GSH} \xrightarrow{\text{peroxidase}} 2 H_2O + \text{GSSG} \quad (\text{oxidized glutathione}) \]
Normally, most of the intracellular glutathione is in its reduced form. Increased intracellular concentrations of \( \text{H}_2\text{O}_2 \) results in a drop in GSH/GSSG ratio, which serves to detect intracellular oxidative stress (Tribble and Jones, 1990).

b) Non-enzymatic antioxidant mechanisms

There are no enzymatic mechanisms to directly protect against \( \cdot \text{OH} \) because of its extremely high reactivity and rapid consumption. Alternatively, cells possess non-enzymatic antioxidant mechanisms which scavenge \( \cdot \text{OH} \). Some of these scavengers are in hydrophilic phase (ascorbate, urate, glutathione) and others in the lipid phase (\( \alpha \)-tocopherol and \( \beta \)-carotene). Vitamin E (\( \alpha \)-tocopherol) is a key \( \cdot \text{OH} \) scavenger and a chain breaking antioxidant in biological membranes (Niki et al., 1988). It reacts with OH or most commonly with lipid peroxyl radicals (LOO') to form the \( \alpha \)-tocopherol radical. Another antioxidant of importance in biological membranes is ubiquinol-10 (coenzyme \( Q_{10} \)). Ubiquinol-10 is as efficient as vitamin-E in chain-breaking antioxidant reactions. It is present in inner mitochondrial membrane where it also functions as an electron carrier in the respiratory chain.

The various defenses are complementary to each other because they metabolize or scavenge different species in different cellular compartments. Dietary or pharmacological enhancement of endogenous antioxidant defenses may be beneficial in disease or aging processes where oxygen radicals are involved.
Cancer

Cancer is the most common term for all malignant tumors. It derives from the Latin for crab, ‘Cancer’ presumably because a cancer adheres to any part that it seizes upon in an obstinate manner like the crab. The growth of cancers is accompanied by progressive infiltration, invasion and destruction of the surrounding tissue. Cancer cells possess an insidious property to migrate from the site where they originate and form masses at distant sites in the body (Weinberg, 1996). Cancer in all forms are causing about 12 percent of deaths throughout the world. In the developed countries, cancer is the second leading cause of death, next to cardiovascular diseases, accounting for 21 percent (2.5 million) of mortality. In developing countries, it ranks third as a cause of death and accounts for 9.5 percent (3.8 million) of all deaths. According to WHO estimates, by the year 2000 the number of cancer deaths may go up to 8 million annually (The World Health Report, 1997). Among men, the leading eight killer sites for cancer are the lungs, stomach, liver, colon-rectum, oesophagus, mouth-pharynx, prostate and lymphoma. In women, they are cancers of the breast, stomach, colon-rectum, cervix, lungs, ovary, oesophagus and liver (WHO, The World Health Report, 1998).

The changes in DNA like base modifications, rearrangement of DNA sequence, miscoding of DNA lesion, gene duplication and the activation of oncogenes may involve the initiation of various cancers (Cavenee and White, 1995). Mutation in several critical genes can lead to tumors (Vogelstein et al., 1989), for example, mutations in the tumor suppressor gene, p53 are found in about half of human tumors. Mutations can convert proto-oncogenes into carcinogenic oncogenes and studies have revealed the presence of human c-oncogenes with several being active.
(Marshall, 1985). These genes were found to be mutated, rearranged or unusually active in many viral and non-viral tumors. Retroviruses lacking c-oncogenes are also oncogenic, giving rise to tumors more slowly than those with v-oncogenes (Weiss, 1986).

Cell division is a critical factor in mutagenesis, because when the cell divides a DNA lesion can give rise to a point mutation, deletion or translocation (Ames et al., 1993; Cohen et al., 1991; Ames and Gold, 1990). With increase in cell division, there is increased risk for cancer, which can be caused by such diverse agents as increased levels of particular hormones (Handerson et al., 1982), excess calories, chronic inflammation or chemicals at doses causing cell division (Cunningham et al., 1994; Columbano et al., 1990; Moalli et al., 1987).

Alcoholic beverages cause inflammation and cirrhosis of the lung and liver (IARC, 1988). Alcohol is an important cause of oral and oesophageal cancer (IARC, 1988) and possibly contributes to colorectal cancer (Giovannucci et al., 1995). Hepatitis B and C viruses are a major cause of chronic inflammation leading to liver cancer, which is one of the most common cancers in Asia and Africa (Tabor and Kobayashi, 1992; Yu et al., 1991). The mutagenic mold toxin aflatoxin, may cause chronic hepatitis infection in liver cancer development (Qian et al., 1994). Some cancer chemotherapeutic drugs, particularly the alkylating agents, cause malignancies, most commonly leukemias, lymphomas and sarcomas (Ellis and Lisher, 1993; Curtis et al., 1992). Potent immuno-suppressive agents such as cyclosporin also increase the risk of a variety of cancers (Ryffel, 1992).

Epidemiological evidence indicates that avoidance of smoking, increased consumption of fruits and vegetables and control of infections
will have a major effect in reducing the rates of cancer (Ames et al., 1995). Other factors include avoidance of intense sun exposure, increase in physical activity and reduction of alcohol consumption (Ames et al., 1995)

**Role of Oxygen Free Radicals in Cancer Development**

Oxygen free radicals are continuously generated in cells exposed to an aerobic environment. A number of endogenous and exogenous cancer risk factors generate free radicals *in vivo* (Swartz, 1972). In recent years convincing evidence has accumulated indicating that oxygen free radicals are indeed a relevant class of carcinogens (Cerutti, 1994; Feig et al., 1994; Guyton and Kensler, 1993). Cancer development is now commonly recognised as a microevolutionary process that requires the cumulative action of multiple events (Klein, 1987). These events can be described in a simplified three-stage model: 1) the induction of DNA mutation in a somatic cell (initiation), 2) the stimulation of tumorigenic expansion of the cell clone (promotion) and 3) the malignant conversion of the tumor into cancer (progression). Oxygen free radicals can stimulate cancer development at all three stages, initiation (Husain et al., 1994), promotion (Nakamura et al., 1988) and progression (Salim, 1993).

With respect to cancer, DNA is considered to be the most important target of ROS (Ames et al., 1995; Feig et al., 1994). Oxidative damage to DNA includes a range of specifically oxidised purines and pyrimidines as well as alkali labile sites and strand breaks, formed directly or by repair processes (Breen and Murphy, 1995; Dizdaroglu, 1994). Floyd et al. (1986) supplied the earliest *in vitro* evidence for a DNA molecular alteration by a free radical-generating tumor promoter. They have successfully isolated a product of oxidatively damaged DNA, 8-OHdG. To
determine whether such radical adducts are involved in carcinogenesis, Kasai et al. (1987) carried out an *in vivo* experiment in which superoxide-producing KBrO₃ was administered to animals. The 8-OHdG was detected in the kidney, confirming possible tumorigenesis by \( \cdot \text{O}_2 \). Additional evidence on DNA damage in human fibroblasts was supplied by Kaneko and Leadon (1986) who detected the production of thymine glycols in DNA by the reaction of N-hydroxy-2-napthalamine. Elevated levels of modified bases in cancerous tissue may be due to the production of large amounts of H₂O₂, which has been found to be characteristic of human tumor cells (Szatrowski and Nathan, 1991). Further, evidence exists that tumor cells have abnormal levels and activities of antioxidant enzymes, such as superoxide dismutase or catalase, leading to accumulation of \( \cdot \text{O}_2 \) and H₂O₂ that induces damage to DNA (Olinski et al., 1992).

In patients with diseases associated with increased risk of cancer, including Fanconi anemia, chronic hepatitis, cystic fibrosis and various autoimmune diseases, studies indicate an increased rate of oxidative DNA damage or in some instances deficient repair (Brown *et al*., 1995; Hagen *et al*., 1994; Shimoda *et al*., 1994; Takeuchi and Morimoto, 1993). Human studies support the experimentally based notion of oxidative DNA damage as an important mutagenic and carcinogenic factor (Loft and Poulsen, 1996). ROS can damage DNA and the division of cells with unrepaired or misrepaired damage leads to mutations. The majority of mutations induced by ROS appear to involve modification of guanine, causing G—\( \rightarrow \)T transversions (Denissenko *et al*., 1996; Du *et al*., 1994; Colapietro *et al*., 1993).

Oxidative stress arises either from the overproduction of ROS or from the deficiency of antioxidant defense or repair mechanisms and results in
reversible or irreversible tissue injury (Dreher and Junod, 1996). An important endogenous cause of chronic oxidative stress is the inflammatory response (Cerutti and Trump, 1991). Activated neutrophils stimulate mutagenesis in vitro (Weitzman and Gordon, 1990) and oxidative stress from chronic inflammation favours cancer development in many organs. Cancer induction by chronic inflammation is frequently observed in ulcerative colitis (Collins et al., 1987). Other examples of inflammation related carcinogenesis are the mesothelioma caused by asbestos deposits (Mossman et al., 1990) and urinary bladder cancer induced by Schistosoma haematobium (Rosin et al., 1994). Important examples of exogenous causes of oxidative stress and their carcinogenic consequences are shown in Table III.

A role of ROS in the development of cancer in humans is further supported by the abundant presence of oxidative DNA modifications in cancer tissue. Thus, the lungs from cancer patients contain 25-75 8-oxodG per 10⁵ deoxyguanosine in the apparently normal tissue and two to three fold higher values in the tumor, in addition to a whole series of other oxidative DNA modifications (Olinski et al., 1992). Smoking, the cause of bronchogenic carcinoma (Doll and Peto, 1981), chronically exposes the bronchial epithelium to ROS (Stone and Pryor, 1994). The urinary excretion of 8-oxodG and related biomarkers suggests that the rate of oxidative DNA modification corresponds up to 10⁴ affected bases per cell/day (Loft et al., 1992, and 1993; Shigenaga et al., 1989; Cathcart et al., 1984).

Free radical induced oxidative base damage most likely represents an event of considerable importance in the progression of breast tumors to the metastatic state and is likely an important etiologic
TABLE III

Major Exogenous Causes of Oxidative Stress Involved in Carcinogenesis

<table>
<thead>
<tr>
<th>Cause of oxidative stress</th>
<th>Oxygen free radicals</th>
<th>Cancer associated with exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco smoke</td>
<td>NO . 'OH</td>
<td>Bronchogenic carcinoma</td>
</tr>
<tr>
<td>Ultraviolet light</td>
<td>'OH , organic radicals</td>
<td>melanoma. skin cancer</td>
</tr>
<tr>
<td>Fatty acids in food</td>
<td>lipid peroxides</td>
<td>colorectal and breast cancer</td>
</tr>
<tr>
<td>Iron and copper ions</td>
<td>'OH</td>
<td>colorectal cancer</td>
</tr>
<tr>
<td>Ethanol</td>
<td>lipid peroxides</td>
<td>hepatocellular and breast cancer</td>
</tr>
</tbody>
</table>

(Adapted from Dreher and Junod. 1996)
factor (Malins et al., 1993 and 1996). The attack of \(^{\cdot}\text{OH}\) on the base structure of breast DNA would be expected to result in genetic instability causing the activation of nuclear oncogenes and the deregulation of tumor suppressor genes such as p53. One study found a 9-fold increase in 8-hydroxyguanine, 8-hydroxyadenine and 2,6-diamino-4-hydroxy-5-formamidopyrimidine in DNA from breast carcinoma compared with control tissue (Malins and Heimanot, 1991).

Exposure to ROS and its cellular production are facts of life. ROS can cause oxidative DNA damage and protein modifications, damage to tumor suppressor genes and enhanced expression of protooncogenes (Cerutti, 1994; Jackson, 1994) and oxidative stress has been shown to induce malignant transformation of cells in culture (Weitzman and Gordon, 1990). However, the development of human cancer depends on many other factors, including the extent of DNA damage, antioxidant defences, repair enzymes, the efficiency of removal of oxidized nucleosides before they are incorporated into DNA and the cytotoxic effects of ROS in large amounts and their growth promoting effects in small amounts (Burdon et al., 1995).

**Systemic Lupus Erythematosus**

Systemic lupus erythematosus is an autoimmune disorder with an annual incidence of 50 to 70/million and a prevalence of 500/million population (Klippel, 1997). Major organ system involvement may occur in the heart, lungs, kidneys and central nervous system and is responsible for most of the mortality caused by the disease. The etiology of the disorder is incompletely understood (Steinberg et al., 1990 & 1991). It is a prototype of spontaneous immune complex disease in which anti-DNA antibody complexes deposit in tissue and induce inflammation (Naparstek...
and Madaio, 1997). Both genetic and environmental factors are believed to contribute to disease (Gourley et al., 1992).

The major serological marker of SLE, antibodies to native DNA, were distinguished by four different groups as early as 1957 (Cepellini et al., 1957; Meisher and Strassie, 1957; Robbins et al., 1957; Seligmann, 1957). There are reports of serum anti-dsDNA antibody levels correlating with the severity of the renal disease in SLE (Wallace et al., 1993). These anti-DNA antibodies found in the sera of patients with SLE are of diverse antigenic specificities (Ali et al., 1991; Pollard et al., 1986), which include components of DNA, its different conformations including modified structures (Alam and Ali, 1992; Hasan et al., 1991). Antigen specific and antigen non-specific factors have been implicated in the autoantibody production in SLE (Klinman et al., 1990; Datta et al., 1987; Klinman; and Steinberg, 1987; Schlomchik et al., 1987). Various serological findings suggest that lupus nephritis results from deposition of DNA-anti-DNA complexes and subsequent complement mediated tissue damage (Nishiya and Hashimoto, 1997; Zouali, 1997; Pisetsky, 1992; Emlen et al., 1986). Pankewyez et al. (1987) demonstrated that eluted immunoglobulins from the kidney of female MLR-lpr/lpr mice with early nephritis was predominantly IgG with antibody activity against DNA. These antibodies were also found to be reactive with multiple nucleic antigens and non-nucleic antigens like cardiolipin, suggesting that polyreactivity might be a distinguishing feature of nephritogenic autoantibodies.

Sequencing of antibody and hybridoma technology have facilitated a lot in understanding the features of anti-DNA antibodies which may impart pathogenicity and/or allow DNA recognition. Anti-DNA IgG which is
cationic in nature produces tissue damage. Putterman et al. (1996) reported that charge and affinity may not predict potential for tissue damage. Recent studies by Swanson et al. (1996) indicated that an anti-ssDNA antibody may be a more potent pathogen compared to an anti-dsDNA antibody. In addition, it has been suggested, that it is not the positive charges which allow pathogenicity but the number of charged (positive and negative) residues within the V region which influences this property (Ohnishi et al., 1994).

Apoptosis is an internally programmed cell death pathway (frequently initiated by extracellular signals), that regulates both T cell and B cell development (Smith et al., 1989; Liu et al., 1989). Accumulating evidence suggests that in the normal situation, immature self reactive thymocytes undergo apoptotic death (negative selection) upon stimulation via the T cell receptors (Von Boehmer, 1986). Emlen et al. (1994) have demonstrated that the rate of apoptosis of lymphocytes derived from SLE patients was 2.4 times faster than seen in lymphocytes from normal controls. The release of intact nucleosomes in excessive amounts during the process of apoptosis may thus provide a source of extracellular nuclear antigens sufficient to drive an immune response and induce DNA antibody production. It has been shown that onset of the autoimmune response in murine models of SLE is characterized by the early emergence of antibodies that recognize conformational epitopes of the nucleosome particles (Burlingame et al., 1993).

There is increasing evidence to suggest a link between autoimmune diseases and cancer (Seda and Alarcon, 1995; Cash and Klippel, 1991; Sela and Shoenfeld, 1988). Patients with SLE have defects in their cellular and humoral immune systems. The basic defect in SLE in humans is
suggested to be a deficiency in suppressor T cell function, which leads to proliferation and hyper-reactivity of B lymphocytes (Fauci et al., 1978). Certain B lymphocytes (CD5+ B cells) have a strong tendency to undergo oligoclonal or monoclonal proliferation and malignant transformation (Hayakawa and Hardy, 1988). Various findings suggest that cancer especially lymphoma and soft tissue sarcoma, is more common in patients with SLE than in general population (Patterson et al., 1992).

The clinical manifestations of SLE are remarkably heterogenous. Patients of SLE also exhibit high lipid profiles, which are known to arise from renal involvement and a nephrotic state (Appel, 1991; Attman and Alaupovic, 1990). Neurological involvement is also common in SLE inflicted individuals (Adelman et al., 1986). More than 60% of patients have CNS manifestations and 30-40% have neuromuscular abnormalities (Omdal et al., 1989). The major causes of death are directly related to the disease and include acute vascular neurologic events, renal failure and coronary artery disease (Klippel, 1997). Medication includes non-steroidal anti inflammatory drugs for mild form of the disease while high doses of corticosteroids and immunosuppressive drugs are needed for chronic SLE patients (Blank et al., 1992).

**Role of ROS in SLE**

Hydroxyl radical, a prominent entity of reactive oxygen species, is known to modify cellular DNA and has been implicated in several human diseases. It has been proposed that in chronic inflammatory diseases such as RA and SLE, highly reactive oxygen species released from activated phagocytic cells at the site of injury may penetrate cellular membranes and react with nuclear DNA (Allan et al., 1988; Lunec et al., 1987; Stollar, 1981). ROS play an important role in the development of
autoimmune diseases particularly SLE (Ahmad et al., 1997; Alam et al., 1993; Ara & Ali, 1993; Blount et al., 1989 & 1990). Oxidative damage to DNA in SLE patients has also been reported (Lunec et al., 1994; Bashir et al., 1993) and supports the role of ROS in the etiopathogenesis of SLE. The presence of oxidative DNA lesions in SLE was immunochemically detected using monoclonal anti-ROS DNA antibody that preferentially recognizes ROS modified epitopes on nucleic acids (Ahmad et al., 1998).

Formation of the altered base 8-OHdG, has been shown to be a very sensitive marker of ROS-induced damage (Aruoma et al., 1989; Kasai and Nishimura, 1984). The presence of 8-OHdG in the urine of normal healthy individuals also affirms that this altered base is a by product of normal oxidative metabolism and likely to be the product of a cellular repair mechanisms. The very low levels of excreted 8-OHdG in the circulating immune complexes of SLE patients, suggests that there is an abnormal repair of damaged DNA in these patients (Lunec et al., 1994). Results from our laboratory suggests a higher specificity of ROS-poly(G) than poly(G) to SLE autoantibodies, which implies that ROS damage may lead to formation of modified guanine moieties in DNA and RNA (Garg and Ali, 1998).

Antigenicity of Nucleotides

Antibodies may also distinguish between nucleosides and nucleotides. With anti-nucleoside antibodies, the large charged phosphate group hinder binding, while for anti-nucleotide antibodies it appears to form part of determinant (Ungar-Waron, 1967; Halloran and Parker, 1966; Erlanger and Beiser, 1964). Seaman et al. (1965) show that antibodies specific for poly(A), poly(C) and poly(I) can be produced in rabbits immunized with
ribopolymer-MBSA complexes. The results of the immunologic studies indicate that nucleotide and DNA-protein conjugates induce the formation of antibodies with nucleotide-protein conjugate (Halloran and Parker, 1966). Immunization with modified nucleosides or nucleotides conjugated to proteins has led to the induction of antibodies (Muller and Rajewsky, 1980). Nucleosides and nucleotides by themselves are not capable of inducing antibodies. So nucleosides are coupled to a carrier protein such as BSA by periodate method and then used for immunization. Whereas nucleotides/deoxynucleotides can be conjugated to carrier protein by carbodiimide method (without cleaving the ribose/deoxyribose ring) utilising the phosphate group present in them. Thus, by retaining the intact ribose/deoxyribose ring the resulting antibodies to nucleotide-BSA conjugates will be specific for DNA or RNA depending on the type of ribose ring present (Chandira Kala and Antony, 1996). Antibodies have been raised against deoxy AMP-BSA, deoxy CMP-BSA and GMP-BSA. While antibodies to deoxy AMP-BSA, deoxy CMP-BSA bind only to DNA (Vaishnav and Antony, 1988, 1989 and 1990), antibodies to GMP-BSA predominantly binds to RNA (Chandira Kala and Antony, 1993).

In human and murine lupus, the most abundant autoantibody populations are those reacting with ssDNA (Eilat, 1986; Schwartz and Stollar, 1985; Munns et al., 1984; Tan, 1982; Andrezejewski et al. 1981). These antibodies can be defined in some detail by an ELISA that employs not only immobilized ss and dsDNA antigens, but nucleotide haptens as well (Weisbart et al., 1983). Generally, such haptens are covalently linked to carrier proteins (e.g. BSA), which by themselves are unreactive with autoantibodies. While anti-ssDNA antibodies recognize individual base moieties with nucleotides (Ballard and Voss, 1985; Munns et al., 1984; Weisbart et al., 1983) one cannot dismiss the possibility
that sequence-specific (deoxy) oligonucleotides are responsible for antibody interaction with nucleic acids (Deutscher and Keene, 1988; Munns et al., 1987; Lee et al., 1981). For example, several monoclonal anti-ssDNA antibodies have been shown to bind with certain TMP-containing polynucleotides but not others (Zouali and Stollar, 1986; Ballard and Voss, 1985; Lee et al., 1981). Munns, et al. (1987) suggested that a significant fraction of anti-ssDNA antibodies in human SLE sera were specific for GMP-enriched oligonucleotides.

Objectives of the Present Study

Systemic lupus erythematosus (SLE) is an autoimmune disorder of unknown etiology and is characterized by the presence of circulating anti-DNA antibodies. Reactive oxygen species have been implicated in a number of human degenerative diseases including cancer and SLE.

In the present study, thymidine 5-monophosphate (TMP) was modified with hydroxyl radical generated by irradiation of hydrogen peroxide at 254 nm. The ROS-TMP was characterized by UV absorption spectra and ion exchange chromatography on DEAE Sephadex A-25 column. TMP being a hapten was linked with BSA via carbodiimide method. TMP-BSA conjugate was modified with hydroxyl radical and characterized by UV spectral analysis, Sephadex G-100 gel chromatography, densitometric scanning and agarose gel electrophoresis.

The antigenicity of ROS-modified TMP-BSA was probed by inducing polyclonal antibodies in rabbits. Induced antibodies were characterized for their fine antigenic specificity with various nucleic acid polymers. Naturally occurring human anti-DNA autoantibodies and the circulating antibodies in sera of various cancer patients were also studied for their recognition to native and ROS modified TMP-BSA conjugates.