Introduction
The sentence "This structure has novel features which are of considerable biological interest" may be one of science's most famous understatements. It appeared in April 1953 in the scientific paper where James Watson and Francis Crick presented the structure of the DNA-helix, the molecule that carries genetic information from one generation to the other. The contributions made by Rosalind Franklin and countless other scientists and researchers have allowed us to uncover the structure and behaviour of DNA and apply that knowledge to better understand genetic diseases and infectious diseases, the immune system, cancer and aging. The most striking feature of Watson and Crick model was antiparallel right handed double helical structure having complementary nucleotide sequence. The biologically important polymorphous form of DNA, the B-DNA, is believed to have a uniform double helical structure in the solid state with the number of residues per turn $n = 10$ and height per residue, $h = 3-4$ Å. These values for the B-form were obtained from models built to explain the crystalline X-ray diffraction patterns of the lithium salt of DNA (Langridge et al., 1960; Arnott, 1970). DNA has an enormous ability to undergo structural changes by twisting, turning and stretching leading to a variety of DNA structures namely A,B,C,D and Z; later differ from the former in having left handed double helical structure instead of the usual right handed helix (Saenger, 1984). The DNA double helix is stabilized primarily by two forces: hydrogen bonds between nucleotides and base stacking interactions among the aromatic nucleobases (Yakovchuk et al., 2006).

**Antigenicity of DNA**

The origin and nature of antigens inducing anti-double stranded DNA antibodies are not known (Stollar, 1992). Although it has been well established that native DNA in B-conformation is not immunogenic (Stollar, 1989; Pisetsky, 1996). Anti-DNA autoantibodies permanently attract the attention of researches. In spite of numerous investigations aimed at determination of their potential targets and role in the disease, origins of anti-DNA autoantibodies and their pathological role remain to be established. Moreover, taking into account of such newly described features of anti-DNA antibodies as the ability to penetrate into the living cell (Alarcon-Segovia et al., 1996), and DNA-hydrolyzing activity (Schuster et al., 1992), one may even assume pathogenic potential of DNA-specific autoantibodies as underestimated. In
general, antibodies that bind DNA do not display strict disease specificity. It is, however, widely accepted that healthy individuals usually express low-affinity antibodies with specificity to single-stranded DNA, while presumably pathogenic high-affinity autoantibodies to double-stranded DNA are frequently over represented on the background of systemic autoimmune abnormalities (Paul et al., 1990). Antibodies to DNA were first described in 1957 (Ceppellini et al., 1957; Holborow et al., 1957; Robbins et al., 1957). They constitute a subgroup of antinuclear antibodies that bind single-stranded DNA, double-stranded DNA or both. They may be IgM antibodies or any of the subclasses of IgG antibodies. In general, tests for IgG complement-fixing antibodies to DNA, especially those that bind double-stranded DNA, have the greatest diagnostic value in patients in whom systemic lupus erythematosus is suspected and in patients with systemic lupus erythematosus the results often correlate with the clinical activity of the disease and with the risk of glomerulonephritis. However, these are not the only types of anti-DNA antibodies that can cause nephritis: some subgroups of IgM antibodies to DNA and some antibodies to single-stranded DNA can probably cause it as well (Rothfield and Stollar, 1967; Schur and Sandson, 1968; Tojo and Friou, 1968). Antibodies that bind exclusively to single-stranded DNA can bind its component bases, nucleosides, nucleotides, oligonucleotides, and ribose-phosphate backbone, all of which are exposed in single strands of DNA. In contrast, antibodies that bind double-stranded DNA bind to the ribose-phosphate backbone, base pairs (deoxyguanosine-deoxycytidine and deoxyadenosine-deoxythymidine), or particular conformations of the double helix (Stollar, 1994; Kalsi et al., 1996). Double stranded DNA exists primarily in a right-handed helical form called B DNA; there is also a left-handed helical form called Z DNA. Some patients with systemic lupus erythematosus have antibodies against both forms, whereas others have antibodies that react preferentially with Z DNA (Stollar, 1994; Herrmann et al., 1995).

Studies of monoclonal antibodies have shown that antibodies that bind exclusively to double-stranded DNA are rare; most antibodies to double-stranded DNA bind both double-stranded DNA and single-stranded DNA. Most normal subjects have IgM antibodies to single-stranded DNA in their serum. These antibodies, which belong to the repertoire of natural autoantibodies, have low affinity for DNA and for several other self-antigens, such as thyroglobulin and myosin.
(Pisetsky et al., 1990; Diamond et al., 1992; Taki et al., 1992). In contrast, IgG antibodies to double stranded DNA are less prevalent in normal subjects and are more likely to include high-affinity subgroups with narrow cross-reactivity (Diamond et al., 1992; Taki et al., 1992). DNA may bind to antibodies that also bind antigens other than naked DNA; such cross-reactivity may be important in causing disease (Schwartz and Stollar, 1985; Brinkman et al., 1990; Burlingame et al., 1994; Reeves et al., 1994; Mohan and Dutta, 1995; Lefkowith et al., 1996). Additional characteristics that contribute to the pathogenicity of antibodies to DNA include their complement-fixing capability, their affinity for DNA and cross-reactive antigens, the charge of the antibody molecule or of the immune complex containing it, and the amino acid sequences of associated proteins (Rothfield and Stollar, 1967; Schur and Sandson, 1968; Tojo and Friou, 1968; Winfield et al., 1977; Mohan and Dutta, 1995).

**Origin of antibodies to DNA**

Several mechanisms can lead to the production of antibodies to DNA. Antibodies to single-stranded and double-stranded DNA are part of the normal repertoire of natural autoantibodies; most of these are low-affinity IgM antibodies that react weakly with several self-antigens (Putterman et al., 1997). However, these natural antibodies can undergo an isotype switch (from IgM to IgG) that increases their potential to be pathogenic. In addition, somatic mutations in the encoding immunoglobulin genes may result in the production of high-affinity IgG antibodies to DNA, the type of antibody most frequently linked to glomerulonephritis in patients with systemic lupus erythematosus (Diamond et al., 1992; Taki et al., 1992; Putterman et al., 1997; Zouali, 1997). Antibodies to DNA can be induced in mice by the injection of irritating chemicals, such as pristane; by stimulation with antigens, such as bacterial DNA, bacterial cell-wall phospholipids and viruses; and by stimulation by complexes of DNA and proteins (Schwartz and Stollar, 1985; Rekvig et al., 1992; Desai et al., 1993; Pyun et al., 1993; Burlingame et al., 1994; Reeves et al., 1994; Mohan and Dutta, 1995; Satoh et al., 1995). These induced antibodies can then be deposited in the glomeruli, where damage may or may not result. Antibodies to DNA, particularly those that cause damage, are more readily induced by immunization of animals with DNA–protein complexes than by immunization with protein-free DNA (Desai et al., 1993). The antigens that initiate the formation of
potentially pathogenic antibodies to DNA may be chromatin (packages of nucleosomes connected by DNA linkers) or nucleosomes (166 to 240 base pairs of DNA wound around an octameric complex of several different types of histone) (Burlingame et al., 1994; Reeves et al., 1994; Mohan and Dutta, 1995, Lefkowith et al., 1996). Evidence of the central role of chromatin and nucleosomes includes the presence of antibodies to these substances in the serum of patients with systemic lupus erythematosus (Burlingame et al., 1994) and the ability of these macromolecular complexes to block the binding of serum immunoglobulins from patients with systemic lupus erythematosus to extracts of glomeruli (Lefkowith et al., 1996). In addition, nucleosome-activated T lymphocytes from patients with systemic lupus erythematosus can help B lymphocytes produce IgG antibodies to DNA (Mohan et al., 1993; Mohan and Dutta, 1995).

The ability to make antibodies to chromatin, nucleosomes and DNA depends in part on genetic susceptibility. For example, in one strain of lupus prone mice, four regions on four different chromosomes are linked to the production of antibodies to chromatin (Theofilopoulos, 1995). In humans a region on chromosome 1 (1q 41–42) may contain a gene or genes predisposing carriers to the production of antibodies to chromatin and predisposing them to systemic lupus erythematosus (Tsao et al., 1997). In addition to DNA-protein complexes, RNA-protein complexes may induce antibodies to DNA. Immunization of rabbits with peptides from the RNA-protein complexes contained in small nuclear RNA particles can induce antibodies to DNA as well as antibodies to small nuclear RNA particles (James et al., 1995). The processes by which the responses of T and B lymphocytes to one antigen expand to include reactivity to additional antigens depend on the degeneration of T-cell antigen receptors so that a single receptor binds more than one peptide-HLA complex (either more than one peptide is bound to one HLA molecule, or one peptide is bound to more than one HLA molecule). Expanded reactivity also depends on determinant spreading, in which expanding populations of T and B cells with different receptors recognize additional regions in the initiating antigen as the immune response matures. Both of these processes occur in human T cells in vitro (Hemmer et al., 1997). Therefore, single T or B cells, initially activated by a single antigen, eventually respond to multiple self and non-self-antigens. Through these mechanisms, multiple exposures to bacterial, viral or chemical antigens and to self antigens (particularly
nucleic acid-protein complexes) can lead to the formation of antibodies to DNA. In people genetically predisposed to systemic lupus erythematosus, some of the antibodies to DNA are pathogenic and the ability to down-regulate the production of those antibodies is defective; therefore, disease results. How do DNA-protein and RNA-protein complexes that should be tolerated by the immune system become immunogenic? In human cells stressed in certain ways, such as by exposure of keratinocytes to ultraviolet light, apoptosis occurs and during apoptosis particles from the nucleus and cytoplasm are packaged in blebs of the cell membrane (Casciola-Rosen and Rosen, 1997). Some of these blebs contain RNA-protein complexes, such as the Ro(SSA) antigen, antibodies to which are associated with rash in patients with subacute cutaneous lupus and in neonates with lupus. Other blebs contain nucleosomes plus small nuclear RNA particles and Ro(SSA). Perhaps the immune system can react to these antigens when they are presented in this manner. In addition, nucleosomes released from dying cells could stimulate the production of antibodies to DNA. A relevant finding is that lymphocytes from patients with systemic lupus erythematosus release increased quantities of nucleosomes (Emlen et al., 1994).

**Origin of pathogenic antibodies to DNA**

Origin of the pathogenic antibodies is shown in Figure 1. Panel A shows a normal immune system, with B cells secreting low-affinity IgM antibodies to DNA, with little or no T-cell help. Panel B shows activation of the normal immune system by a self-antigen (DNA–protein complexes, shown as chromatin or nucleosomes) and an environmental antigen (shown as bacteria). These antigens are taken up by professional antigen-presenting cells or bind to antibodies (induced by the antigens) on the surface of the B cells. Both antigen-presenting cells and B cells process the antigens into peptides and present them to T cells as complexes with surface HLA molecules of the cells (Panel C). In addition, peptides from the immunoglobulin molecules themselves are presented (by the B cell on the left of Panel C). If second-signal molecules on the T cells are also linked with their ligands on B cells or antigen-presenting cells (the CD 40 and CTLA 4 systems are shown), the T cells become activated. By release of cytokines and by contact with B cells, those helper T cells cause the B cells to secrete high-affinity IgG antibodies to DNA, and disease may result.
Fig. 1 Origin of pathogenic antibodies to DNA

The role of anti-DNA antibodies in disease pathogenesis

Some antibodies to double-stranded DNA cause glomerulonephritis by forming complexes with DNA that are passively trapped in the glomeruli, whereas others cause glomerulonephritis by direct attachment to glomerular structures. Antibodies to DNA can be eluted from diseased glomeruli and other tissues in some patients with systemic lupus erythematosus (Winfield et al., 1977), suggesting that these antibodies cause tissue damage. High titres of serum antibodies to double-stranded DNA were correlated with the presence of active systemic lupus erythematosus and especially with glomerulonephritis in many studies (Rothfield and Stollar, 1967; Schur and Sandson, 1968; Weinstein et al., 1983; ter-Borg et al., 1990; Smeenk et al., 1991; Bootsma et al., 1995; Quismorio, 1997). Serum samples from some patients with lupus glomerulonephritis (and also from some without nephritis) contain immunoglobulins that bind to extracts of human glomerular basement membrane (Lefkowith et al., 1996); pretreatment of extracts with DNase abolishes much of the reactivity. This suggests that antibodies to DNA cause nephritis by binding to DNA planted in components of glomerular basement membrane. In normal mice, lupus like glomerulonephritis can be induced by the transfer of monoclonal mouse antibodies to DNA (Ohnishi et al., 1994), or by the introduction into the germ line of genes encoding the heavy and light chains of a murine IgG antibody to double-stranded DNA (Tsao and Hahn, 1992). Some human monoclonal antibodies to DNA produced by B-cell hybridomas transplanted into mice with severe combined immunodeficiency cause glomerulonephritis (Ehrenstein et al., 1995). It is not clear what features distinguish pathogenic from nonpathogenic antibodies to DNA. Complement fixation may be essential for tissue damage; thus IgG1 and IgG3, which fix complement, are enriched in pathogenic antibodies (Rothfield and Stollar, 1967; Schur and Sandson, 1968; Tojo and Friou, 1968). However, IgG2 antibodies as well as IgG1, IgG3 and IgM antibodies to DNA are found in glomerular lesions of patients with lupus glomerulonephritis (Imai et al., 1997). Among blacks in the United States and whites in the Netherlands, subjects with lupus glomerulonephritis are more likely than normal subjects to have alleles for FcRIIa that bind the Fc portions of IgG2 more weakly than do the Fc receptors in normal subjects (Duits et al., 1995; Salmon et al., 1996).
In blacks and whites in the United States, inheritance of an allele encoding FcRIIIa receptors predisposes them to systemic lupus erythematosus and its nephritis (Wu et al., 1997). Taken together these data suggest that a decrease in the ability of mesangial cells and cells of monocyte-macrophage lineage to bind or phagocytize immune complexes containing IgG1, IgG2 or IgG3 predisposes such people to lupus nephritis. Cationic charge gives an antibody to DNA a pathogenic advantage (Suzuki et al., 1993), probably because the antibody binds to negatively charged molecules in glomerular basement membrane. High affinity for DNA also probably gives a pathogenic advantage to an antibody, at least in terms of inducing glomerulonephritis. Immunoglobulins deposited in lupus lesions are enriched in idiotypes that are commonly present in antibodies to DNA; these idiotypes may serve as markers of pathogenic antibodies (Kalanian et al., 1989; Isenberg et al., 1990; Suzuki et al., 1991; Shoenfeld, 1994). Some antibodies to DNA from patients with systemic lupus erythematosus bind to membranes of living cells in vitro, penetrate the cells (probably through the myosin in cell membranes), and bind to cytoplasmic or nuclear structures (Alarcon-Segovia and Llorente, 1983; Koren et al., 1995; Yanase et al., 1997). The consequences of this cell penetration are not known, but it could influence cell proliferation, protein synthesis and apoptosis.

The ability of antibodies to DNA to bind additional antigens in glomerular basement membrane (such as C1q or nucleosomes bound to type IV collagen, for example) or tissue components of glomeruli or vessel walls (such as laminin or heparan sulfate) may be a major determinant of pathogenicity (Brinkman et al., 1990; Ohnishi et al., 1994; van Bruggen et al., 1996; Lefkowith et al., 1996). Antibodies to DNA that bind nucleosomes seem to be particularly pathogenic, because they can bind heparan sulfate (probably because of the positive charges on nucleosomes), and they can bind nucleosomes trapped in type IV collagen in glomerular basement membrane (Burlingame et al., 1994, Reeves et al., 1994, Mohan and Dutta, 1995; Lefkowith et al., 1996; van Bruggen et al., 1996). Binding of serum immunoglobulin from patients with systemic lupus erythematosus to extracts of glomerular basement membrane is inhibited more effectively by nucleosomes than by protein-free DNA. Thus, there are several mechanisms by which antibodies to DNA can damage glomeruli and probably other tissues as well.
Regulation of production of anti-DNA antibodies

The production of IgG antibodies to DNA requires interactions between B cells, which produce the antibodies and helper T cells, which further activate the synthesis and secretion of antibodies by B cells (Linker-Israeli et al., 1990; Pisetsky et al., 1990; Mohan et al., 1993). In patients with systemic lupus erythematosus, both CD4 (which normally act as helpers) and CD8 T cells activate the synthesis and secretion of antibodies (Linker-Israeli et al., 1990; Mohan et al., 1993). Therefore, cells that normally suppress the activation of B cells, the CD8 T cells and natural killer cells, are defective in their suppressive activity. The hyperactivity of T-cell help is well illustrated in mice and humans with lupus, in which peptides processed from autoantibodies to DNA, activate helper T cells, a process that in turn causes increased synthesis of pathogenic antibodies to DNA (Singh et al., 1995; Williams et al., 1995). In normal mice, in contrast, T cells are not spontaneously activated by immunoglobulin-derived peptides and the mice have regulatory cells that suppress the synthesis of antibodies to DNA (Singh et al., 1995). Clearing of immune complexes by phagocytic cells is also defective in patients with systemic lupus erythematosus (Salmon et al., 1996; Gauthier and Emlen, 1997). This is due in part to reduced numbers of CR1 receptors for complement on cell surfaces (Krych et al., 1992). Defective clearance also may be due to inadequate phagocytosis of IgG2 and IgG3 containing complexes (Duits et al., 1995; Salmon et al., 1996; Wu et al., 1997). The idiotypic networks that prevent overproduction of antibodies in normal subjects are probably defective in patients with systemic lupus erythematosus (Abdou et al., 1981; Williams et al., 1995).

Environmental pollutants

Chemical carcinogens are substances or mixtures which have the potential to induce cancer to humans under certain conditions and for prolonged or excessive periods of exposure. Chemical carcinogens can be natural chemicals, synthetic compounds or mixtures of both, that are produced or used for industrial, agricultural or commercial purposes. Carcinogens can cause cancer by direct action in the cellular DNA or through mechanisms that generate chemical species (such as free radicals, reactive oxygen species, and carcinogenic metabolites) which enter the cell nucleolus.
causing mutations to cellular DNA. Chemically-induced cancer generally develops many years after exposure to a carcinogenic agent. A latency period of as much as thirty years has been observed between exposures to asbestos fibres (a known carcinogenic agent), and incidence of lung cancer (Barrett and Wiseman, 1987; Weinstein, 1988).

Carcinogenesis is a multistep process that proceeds through multiple discernible stages, including initiation, promotion, and progression. The transition between these stages is driven by different environmental and endogenous factors and involves different mechanisms and genetic elements. Several types of chemicals initiate the carcinogenic process by yielding highly reactive species that bind covalently to cellular DNA. These carcinogenic agents distort the conformation of DNA and its functions during DNA replication and transcription. These changes have implications with respect to oncogene activation, DNA amplification, gene transposition and chromosome translocation. Carcinogenic chemicals may influence the carcinogenic process by mutational activation of protooncogenes and/or inactivation of tumor suppression genes. In addition, chemical carcinogens may act on non-mutational processes such as the clonal expansion of pre-malignant cells. The carcinogenic risk of specific chemical carcinogens is a composite of its effects on multiple genetic and epigenetic processes (Barrett and Shelby, 1992; Wogan et al., 2004). Exogenous chemical carcinogenesis is an extremely complex multi-factorial process during which gene-environment interactions involving chronic exposure to chemical carcinogens and polymorphisms of cancer susceptibility genes add further complexity. These exogenous chemical carcinogens could be major contributors to human cancer (Irigaray and Belpomme, 2009). Chemicals related to environmental pollution appear to be of critical importance in inducing cancer, such as occupational cancers. Scientists have established that outdoor air pollution (suspended particulate matter associated with other carcinogenic chemicals), indoor air pollution (environmental tobacco smoke, formaldehyde, benzene and 1,3-butadiene), food pollution (food additives, pesticide residues, dioxins, organochlorines) and other chemical pollutants (such as metals, metalloids, pharmaceutical medicines, cosmetics, etc) may contribute to malignant neoplasms in humans (Belpomme et al., 2007). In recent years, epidemiologists and cancer specialists agree that environmental factors play an important part in carcinogenesis. But, it is evident to them that especially
lifestyle factors (tobacco smoking, diet, alcohol consumption, obesity, sedentary life and other known lifestyle factors (excessive exposure to sunlight, viruses, sexual life, hormonal changes, etc.) are contributing to a major proportion of human cancers (Irigaray et al., 2007). Occupational cancers are known to cause, approximately, 4-5% of human cancers, but in recent years new health and safety regulations in the working environment and the substitution and/or restriction of many known chemical carcinogens reduced substantially the risk of exposure to workers (Montesano and Hall, 2001; Clapp et al., 2008).

Carcinogenicity of environmental tobacco smoke

Amongst the environmental hazards that are known to cause carcinogenesis, tobacco smoke is a fore runner. In 1964, the Surgeon General warned in his first report on ‘Smoking and Health’ that active smoking causes disease and death. Today, the health risks of active smoking are well known. It also has become obvious that exposure to environmental tobacco smoke (ETS), i.e. ‘passive’ or involuntary smoking in the workplace, public places or in the home is more than a simple nuisance to non-smokers. Of particular concern is the question whether ETS increases the risk of non smokers to develop lung cancer. In 1992, the US Environmental Protection Agency analysed the available evidence on adverse respiratory health effects, including lung cancer, in non-smoking spouses of smokers and concluded that ETS is a human carcinogen. From available exposure data and anticipated size of the US population exposed to ETS, it was calculated that ETS might be responsible for ~3000 lung cancers per year in non-smokers aged 35 and over. Involuntary exposure to ETS must be added to voluntary smoking as a risk factor. ETS is a mixture of cigarette sidestream smoke (SS) and cigarette mainstream smoke (MS). It contains 15% of MS, the smoke first inhaled by an active smoker and then exhaled; while briefly retained in the lung, the smoke is scrubbed of some of its constituents, most notably nicotine, CO and much of the particulate matter. Approximately 85% of ETS is SS, the smoke curling off the end of a lit cigarette between puffs. SS is generated at lower burning temperatures than is MS, has a different chemical composition and ages rapidly. Most notably, carcinogens in cigarette smoke are found in higher concentrations in SS than in MS. On the other hand, non smokers are exposed to ETS
diluted by several orders of magnitude compared to the MS inhaled by active smokers.

The attributable risk of cigarette smoking for bladder cancer is at least 50% (Silverman et al., 1992). Smoking black tobacco cigarettes rather than blonde tobacco cigarettes is a risk factor for bladder cancer (Vineis et al., 1984; Iscovich et al., 1987; Clavel et al., 1989; De Stefani et al., 1991). Chemical analysis of smoke from each of the two types of tobacco has revealed that black tobacco produces higher amounts of aromatic amines (Patrianakos and Hoffman, 1979). Smokers of black tobacco cigarettes have significantly elevated levels of aromatic amine hemoglobin adduct.

In smokers, levels of DNA adducts, either adducts to complex mixtures of aromatic/hydrophobic compounds or to other substances present in tobacco smoke, have been found to be elevated in numerous human tissues, as shown by abundant scientific evidence (Phillips, 2002). These data are not restricted to tissues directly exposed to tobacco smoke but evidence is accumulating for other organs as well. Overall, DNA adduct measurements can be viewed as an integrated bio-index of exposure, metabolism and metabolic activation of carcinogens in smokers (Phillips, 2002). There have been considerably fewer biomarker studies using DNA, or protein adducts as surrogates, done in non-smokers exposed to ETS, and the results obtained are more variable in comparison to smokers. There are studies showing increased levels of DNA adducts in involuntary smokers but those with negative results can also be found. The types of adducts found in involuntary smokers include benzo [a] pyrene diol-epoxide (BPDE) DNA adducts detected by immunoassays, 32P-postlabelled bulky adducts, and oxidative damage to DNA. The variability in findings may be related to the relatively small differences observed in adduct levels between smokers and non-smokers which is likely at least in part to be due to exposure to similar compounds from other sources. However, some studies have found ETS exposure as a significant determinant of aromatic DNA adducts. In non-smoking Greek subjects with self-reported ETS exposure, adduct levels were found to parallel plasma cotinine concentrations, the levels being higher in men compared to women (Georgiadis et al., 2001). Also oxidative DNA damage has been observed in involuntary smokers, similar to a positive finding in smokers (Howard et al., 1998). In line with this finding, decreased levels of components of the antioxidant defense
system were reported for involuntary smokers (Yildiz et al., 2002). Protein adducts, which can be regarded as an indirect alternative to DNA binding, distinguish not only smokers from non-smokers but also ETS exposed non-smokers from non-exposed individuals.

**In vitro studies on damage to DNA by environmental tobacco smoke**

Solutions of tar isolated from ETS, similar to cigarette tar from MS, induced single-strand DNA breaks in rat thymocytes. The DNA damage increased with increasing ETS tar concentrations until a plateau was reached (Bermudez et al., 1994). Another study used the Comet assay (single cell gel electrophoresis) and investigated the genotoxic effects of the particulate and gaseous phases of sidestream smoke after aging and dilution (Wolz et al., 2002). Cells from a human bronchial epithelial cell line were grown in a culture system allowing direct exposure of the cells to different concentrations of airborne ETS. Aged and diluted SS caused a dose-dependent induction of DNA strand breaks. DNA damage was also observed in bronchial epithelial cells treated with 1:10 diluted SS.

Over the decades numerous studies have investigated the genotoxicity of SS or ETS collected from indoor environments under controlled or real-life conditions as particulate matter and found these forms of tobacco smoke to be mutagenic in the traditional Salmonella/plate incorporation assay (the Ames assay) or its modification utilizing the microsuspension method (Husgafvel-Pursiainen et al., 1986, Lo froth and Lazaridis, 1986). In addition, some of the studies have reported on the bacterial mutagenicity of SS and ETS also in the absence of metabolic activation (Ling et al., 1987). These studies have since been replicated in more recent investigations. Using the salmonella/microsome assay, SS condensates from high-tar and low-tar cigarettes were found to induce dose-dependent mutagenicity. In fact, mutagenic activity of the condensate from low-tar cigarettes was 20% more mutagenic than that from high-tar cigarettes (Chortyk and Chamberlain, 1990).

**In vivo studies on damage to DNA by environmental tobacco smoke**

There are several reports of increased levels of smoke-related bulky adducts or oxidative damage (8-oxo-dG) to nuclear DNA. This has been observed in various tissues from rodents treated in experimental conditions where whole-body exposure to
MS, aged and diluted SS, or a mixture of SS and MS were used to mimic ETS exposure (Lee et al., 1992, 1993). From these, a series of experiments showed a significant increase in smoking-related DNA adducts measured by synchronous fluorescence spectrophotometry and 32P-postlabelling in DNA from tracheal epithelium, lungs, heart, bronchoalveolar lavage cells, bladder and testis of rats or mice, with a diagonal radioactive zone detected in 32P-postlabelling; many of the tissues examined showed a time dependent increase in adduct levels (Izzotti et al., 1999). Also, oxidative DNA damage was found to be significantly increased in the lungs of exposed rats (Izzotti et al., 2001). In an experiment, significantly increased levels of adducts to mitochondrial DNA were detected by 32P-postlabelling, after whole-body exposure of rats to MS for 100 consecutive days (Balansky et al., 1996). Adducts to mtDNA were significantly higher than adducts to nuclear DNA both in the liver and in the lungs.

**Arylamines**

Arylamines (aromatic amines) are a group of chemicals that contain both aromatic hydrocarbon and amine entities. The basic structural representative of arylamines is aniline, which has one aromatic ring and a primary amine substituent. Arylamines may also contain more than one aromatic ring (e.g. benzdine and 4-ABP) and/or more than one amine (e.g. benzdine, toluendiamine). The structures include chemicals with one or more of the aromatic rings. The 4,4′-methylenebis(2-chloroaniline) (MOCA) has two amine groups. All of the amines in these structures are primary amines. Based on the chemical structures, the physical properties of various arylamines can be predicted. Hydrophobic and hydrophilic properties depend on the number and polarity of the substituents as well as the number of aromatic rings. For example, water solubility of some arylamines range from 36 g/l (aniline) to 0.2 g/l (4-ABP). In a quantitative structure-activity study, Benign and Passerine, (2001) developed a model that predicts the carcinogenicity of a given arylamine structure based on amine substitution and number of aromatic rings. In this study, drophobicity (as measured by the octanol/water partition coefficient, Log Pow) was the best predictor of the potency of the carcinogenic aromatic amines, whereas mainly electronic reactivity and steric characteristics separated carcinogens from non-carcinogens. Hydrophobic aromatic amines more easily cross biological membranes
and interact with enzymes and macromolecules. In addition, the positioning of the main substituents (ortho, or para) can predict the relative potency of a given aromatic amine (Talaska, 2003). For example, if the amine group is substituted in the β or 2 positions of the polycyclic aromatic compounds (e.g. β-naphthylamine) or in the para position of phenyl or polyphenyl compounds, the chemical is more carcinogenic than amine substitutions at other positions. An example is 4-ABP, which is more carcinogenic than 3-ABP and 2-ABP (Talaska, 2003). This is due to the fact that compounds substituted in ortho or para positions are primarily N-oxidized and activated whereas compounds substituted in other positions are mainly ring oxidized and detoxified. The spatial characteristics and molecular weight plays a role in determining if the chemical is absorbed by the dermal route. In general, increasing the amine substitution increases the chance of dermal absorption and faster distribution to the tissues (Benigni and Passerine, 2001).

4-Aminobiphenyl

4-ABP belongs to the class of chemicals known as aromatic amines, which are capable of inducing a variety of toxic effects, including cancer and methemoglobinemia (Miller, 1970). Human exposure to aromatic amines occurs from both occupational and environmental sources. Aromatic amines occur in occupational settings such as industrial production of benzidine-based dyes and for use in textiles, leather, and other applications (Parkes and Evans, 1984). In addition, various forms of cooked food have also recently been identified as sources of these mutagenic agents, including the related class of compounds termed heterocyclic aromatic amines.

Non-occupational sources of aromatic amine exposure have also been identified. Cigarette smoke is the most significant of these. Sidestream (SS) emitted from the tobacco products constitutes a major source of environmental pollutants. Mainstream smoke (MS) which escapes to the environment from the mouthpiece of the cigarette, cigar, or pipe can also contribute to indoor air pollution (Kuller et al., 1986). ABP is a constituent of cigarette smoke and is found in the particulate phase of SS and MS.

At present, it is believed that the main route of exposure to ABP is cigarette smoke. This source of exposure requires inhalation as the route of entrance to the
body. Occupational exposure to ABP, however mainly involves adsorption through the skin as the route of entry (Underwood, 1977). ABP is found in MS at level of 1-5 ng per cigarette (Patrianakos and Hofmann, 1979). Estimation of systemic dose in smokers, via the haemoglobin adduct formed by an ABP metabolite which indicates a value of 5pg adduct /g of haemoglobin per each cigarette smoke per day, suggests that a considerable fraction inhaled by a smoker is absorbed (Skipper and Tannenbaum, 1990).

In animal studies, both skin and inhalation, as routes of exposure to aromatic amines, have been strongly lined to the development of bladder cancer (Underwood, 1977). Epidemiological studies have confirmed the link between occupational exposure to benzidine-based dyes and bladder cancer (Parkes and Evans, 1984). Industrial uses of other aromatic amines, such as ABP and 2-amino naphthalene, have been also linked to human bladder cancer (Vineis, 1994). Epidemiological studies have indicated the relative risk ratio of bladder cancer to smokers over that of non-smokers to be in the range of 1.5 to 3.0; moreover, higher relative risks have also been reported (Bryant et al., 1987).

In addition to bladder cancer, exposure to aromatic amines, including ABP, has been associated with carcinogenesis in other organs/tissues, such as breast and colon. Tobacco-related carcinogen DNA adducts has been detected in human breast tissue. Tobacco smoke-derived aromatic amines such as ABP and 2-aminonaphthalene could be mutagenic and carcinogenic in the breasts of humans who smoke, as these compounds have been shown to be metabolically activated and to cause DNA damage in human breast epithelial cells in culture, and have been shown to induce mammary tumors in laboratory animals (Ambrosone et al., 1996).

Thus, one of the major concerns to humans exposed to aromatic amines is chemical-induced carcinogenesis. The aromatic amines may exert other toxicities, however, depending on the dose.

**Metabolic activation of 4-ABP to N-OH-AABP**

The non-bonding pair of electrons on nitrogen of ABP can be delocalized in to the aromatic system by resonance and are relatively unreactive thus ABP need to
undergo biotransformation with the help of xenobiotic metabolizing enzymes to yield reactive metabolites, which are capable of binding covalently to DNA and forming promutagenic DNA adducts (Kadlubar et al., 1982) The primary step in the metabolic activation of 4-ABP is its N-oxidation by specific cytochrome P450 (CYP1A2) in hepatic tissues (Butler et al., 1989). The resulting hydroxyarylamine, N-OH-ABP is also generated both via N-methylation or peroxidation but to a lesser extent (Kadlubar et al., 1988; Ziegler et al., 1988). Further metabolism of N-OH-ABP involves conjugation of the N-hydroxyl function with acetate, sulfate, or glucuronate (King, 1974; Kadlubar et al., 1977; Beland and Kadlubar, 1985; Lai et al., 1985). Reactions of N-OH-ABP are catalysed by phase II enzymes, aryl amine N-Acetyl Transferases 1 (NAT1) and NAT2 to form N-OH-4-Acetyl-ABP (N-OH-AABP) (Minchin et al., 1992). N-OH-AABP is considered the metabolite of ABP most likely to form nitrenium ion, which is an electrophilic species that reacts with DNA to yield mutagenic adducts perhaps even within uroepithelium. This nitrenium ion formation could result either from hydrolysis of N-OH-AABP, or by solvolysis after conjugation with a phase II enzyme such as sulfotransferases or glucuronuyltransferase. This hydroxamic acid (i.e N-OH-AABP) is considered to be "proximate" carcinogen (Miller et al., 1961), i.e., metabolic intermediates between the parent carcinogens, also termed precarcinogens (Miller, 1970) and the "ultimate" carcinogens (Miller and Miller, 1966). The ultimate carcinogen is the metabolite whose interaction with tissue constituents is essential for the initiation of the carcinogenic process. So here in this case ultimate carcinogen is an electrophilic reagent i.e the nitrenium ion, capable of binding to macromolecules such as proteins and nucleic acids to form adduct. Schematic representation of bioactivation pathway of aryl amines is depicted in Figure 2.

In fact, ABP–DNA adducts have been detected in the exfoliated urothelial cells of cigarette smokers and their levels correlate with levels of 4-aminobiphenyl–haemoglobin adducts in the same subjects (Bryant et al., 1987; Vineis et al., 1990; Talaska et al., 1991; Curigliano et al., 1996). Furthermore, several DNA adducts are formed upon exposure of human bladder cells to N-OH-ABP, N-OH-AABP and N-OAc-AABP (Hatcher and Swaminathan, 1995; Swaminathan and Reznikoff, 2000; Torino et al., 2001). However, the major (80%) DNA adduct formed has been identified as N-(deoxyguanosin-8-yl)-4-aminobiphenyl (dG-C8-ABP) (Torino et al., 2001).
Fig. 2  Arylamine detoxification and bioactivation pathways. CYP1A2, cytochrome P450 1A2; NAT, arylamine N-acetyltransferase; SULT, sulfotransferase; UGT, UDP-glucuronosyltransferase. (pathway A and D, N-acetylation; B and C, N-hydroxylation; E, O-acetylation; F and J, O-sulfation; G, N-glucuronidation; H,N, O-transacetylation; I, O-glucuronidation).

In addition to covalent adduct formation, these arylamines also cause oxidative DNA damage producing reactive oxygen species (Burger et al., 2001) that ultimately generate DNA strand breaks and base modifications such as 8-oxo-guanine and related products (Dizdaroglu, 1992; Marnett, 2000).

**Genotoxicity of N-OH-AABP**

Because one of the *in vivo* target cell types for ABP is human uroepithelial cells (HUC), an *in vitro* system was developed using human urothelial cells (HUC) to study the role of ABP in bladder cancer (Reznikoff et al., 1992; Swaminathan and Reznikoff, 2000). The system contains two proximate metabolites of ABP, N-hydroxy-4-aminobiphenyl (N-OH-ABP) and N-hydroxy-4-acetylaminobiphenyl (N-OH-AABP) (Burger et al., 2001), which cause DNA damage resulting in neoplastic transformation and mutation (Bookland et al., 1992; Hatcher et al., 1992; Swaminathan and Reznikoff, 1992; Swaminathan et al., 1994). Through 32P-postlabeling analysis of bladder cells exposed to N-OH-ABP or N-OH-AABP in culture, two primary DNA adducts were revealed. They were identified as N-(deoxyguanosin-8-yl)-4-aminobiphenyl (dG-C8-ABP) and N-(deoxyadenosin-8-yl)-4-aminobiphenyl (dA-C8-ABP) (Frederickson et al., 1992; Hatcher and Swaminathan, 1995a&b). In addition, a minor adduct, 3-(deoxyguanosin-N2-yl)-4-acetylaminobiphenyl (dG-N2-AABP) was identified from the horseradish peroxidase mediated bioactivation of N-OH-AABP (Hatcher and Swaminathan, 1995b). The dG-N2-AABP was found in relatively smaller amounts and it displayed different chromatographic mobility compared to that of dG-C8-ABP and dA-C8-ABP (Hatcher and Swaminathan, 1995b; Torino et al., 2001). In HUC, the deacetylated adducts dG-C8-ABP and dA-C8-ABP are produced by the predominant acetyltransferase-mediated activation of N-OH-AABP. In addition, urothelial cells contain a number of peroxidases, including prostaglandin endoperoxide synthetase (Wise et al., 1984; Boyd and Eling, 1985). These peroxidases could convert N-OH-AABP to reactive electrophiles, that is, arylnitrenium and arylamidonium ions, the latter of which could possibly interact with DNA bases to generate the covalent adducts containing the acetyl group.
In addition to generate covalent adducts containing the acetyl moiety, described earlier, the peroxidative metabolism may generate free radicals that could interact with oxygen to produce superoxide anions. These superoxide anions could ultimately generate hydroxyl radicals and the associated reactive oxygen species (ROS) (Marnett, 2000). The ROS could attack DNA bases, causing oxidative damage to produce modified bases, such as 8-oxo-29-deoxyguanosine (8-oxo-dG) (Ward et al., 1987). In fact, the 8-oxo-dG has been shown to cause miscoding by DNA polymerase in vitro, as well as inducing G→T transversions. Such mutations are commonly seen in select oncogenes and tumor suppressor genes following oxidative DNA damage (Shibutani et al., 1991; Cheng et al., 1992; Moriya, 1993; Le Page et al., 1995; Hussain and Harris, 1998). The ROS is presumably generated by endogenous peroxidases, and the DNA damage caused by ROS might be partly responsible for initiation of bladder neoplasia. These findings, along with the earlier reports on the distribution of acetyltransferase-mediated activation of N-hydroxy metabolites of ABP (Frederickson et al., 1992), clearly show that the human bladder epithelia have the necessary enzymes to generate reactive species from N-hydroxyarylamine derivatives. The reactions catalyzed by these enzymes may play a determinate role in the mutagenic and carcinogenic effects of arylamines. The N-hydroxy metabolites of ABP induce mutations in the hypoxanthine guanine phosphoribosyl transferase gene of human uroepithelia and also cause neoplastic transformation and progression in vitro (Bookland et al., 1992). Earlier published reports on chemical interaction of the sulfate and acetate esters of arylhydroxamic acids of acetylaminofluorene with various bases showed a preferential interaction with deoxyguanosine compared to the other bases (Kriek and Westra, 1979; Kadlubar et al., 1988). The N-OAc-AABP and related esters of N-OH-AABP also show a similar preference for interaction with deoxyguanosine, although the yields were relatively lower compared to those of the acetylaminofluorene derivatives (Bartsch et al., 1972). Several investigators reported the formation of N-acetoxyarylhydroxamic acids from arylhydroxamic acids, including N-OH-AABP following catalysis by peroxidases (Bartsch et al., 1972; Corbett and Corbett, 1987). The 8-oxo-dG is not always the most abundant oxidized DNA product, but it is biologically very active. It causes miscoding by DNA polymerase in vitro, and induces mutations in bacterial and mammalian cells (Le Page et al., 1995; Shibutani et al., 1991; Cheng et al., 1992).
In addition to the above-illustrated effects on the formation of ROS and 8-hydroxy-guanine products, the TCC10 cultures exposed to N-OH-AABP also revealed significantly increased levels of DNA strand breaks compared to those of the untreated controls, when measured using alkaline elution methods (Burger et al., 2001). These data lend support for the formation of hydroxyl radicals. The importance of acetyltransferases in the formation of covalent DNA adducts has been well established. ROS, produced during the peroxidase-mediated activation of N-hydroxy metabolites of ABP, might also contribute to urothelial DNA damage as well as to the initiation of neoplasia in human urinary bladders.

Cancer

The term cancer is defined as uncontrolled / contiguous growth of tissue comprising of unrippened, immature or undifferentiated cells of recognizable morphologic appearances, due to failure of host tissue / organ to produce ripened cells for organized repair. It is a consequence of protracted sequence of events in the bioenvironment in-vivo, under influences of the genetic apparatus which has been identified to be incorporating sets of genes like oncogenes and tumor suppressor genes, responsible for giving way to multi-step carcinogenesis.

Cancer itself is a multi step process passing through the following three stages: initiation, promotion, and progression (Hennings et al., 1993) (Fig. 3). Initiation involves an irreversible genetic change, usually a mutation in a single gene. Promotion is generally associated with increased proliferation of initiated cells, which increases the population of initiated cells. Progression is the accumulation of more genetic mutations that lead to the acquisition of the malignant or invasive phenotype. Three important steps involved in initiation are carcinogen metabolism, DNA repair, and cell proliferation. Many chemical agents must be metabolically activated before they become carcinogenic. Most carcinogens, or their active metabolites, are strong electrophiles and bind to DNA to form adducts that must be removed by DNA repair mechanisms. Hence, DNA repair is essential to reverse adduct formation and to prevent DNA damage. Failure to repair chemical adducts, followed by cell proliferation, results in permanent alterations or mutation(s) in the genome that can lead to oncogene activation or inactivation of tumor suppressor genes. Promotion is a
Fig. 3  Multi-stage chemical carcinogenesis. Four stages include: tumor initiation, tumor promotion, malignant conversion, and tumor progression. Activation of proto-oncogenes and inactivation of tumor suppressor genes are mutational events that occur as the result of covalent damage to DNA caused by chemical exposures. The accumulation of mutations and not necessarily the order in which they occur constitutes multistage carcinogenesis.

A reversible process in which chemical agents stimulate proliferation of initiated cells. Typically, promoting agents are non-genotoxic, that is they are unable to form DNA adducts or cause DNA damage but are able to stimulate cell proliferation. Hence, exposure to tumor promoting agents results in rapid growth of the initiated cells and the eventual formation of non-invasive tumors.

Progression refers to the process of acquiring additional mutations that lead to malignancy and metastasis. Many initiating agents can also lead to tumor progression, strong support for the notion that further mutations are needed for cells to acquire the phenotypic characteristics of malignant tumor cells. Damage to DNA and the genetic mutations that can result from them are a central theme in carcinogenesis. Hence, the environmental factors that cause DNA damage are of great interest. Environmental agents that can cause DNA damage include ionizing radiation, ultraviolet (UV) light, and chemical agents (Bertram, 2000). Some of the DNA lesions that can result include single-strand breaks, double-strand breaks, base alterations, cross-links, insertion of incorrect bases, and addition/deletion of DNA sequences. Cells have evolved several different repair mechanisms that can reverse the lesions caused by these agents which has been extensively reviewed (Friedberg et al., 1995). The metabolic processing of environmental carcinogens is also of key importance because this can determine the extent and duration to which an organism is exposed to a carcinogen. Phase I and phase II metabolizing enzymes play important roles in the metabolic activation and detoxification of carcinogenic agents. The phase I enzymes include monoxygenases, dehydrogenases, esterases, reductases, and oxidases. These enzymes introduce functional groups on the substrate. The most important superfamily of the phase I enzymes are the cytochrome P450 monoxygenases, which metabolize polyaromatic hydrocarbons, aromatic amines, heterocyclic amines, and nitrosamines. Phase II metabolizing enzymes are important for the detoxification and excretion of carcinogens. Some examples include epoxide hydrase, glutathione-S-transferase, and uridine 5-diphosphate (UDP) glucuronide transferase. There are also some direct acting carcinogens that do not require metabolic activation. These include nitrogen mustard, dimethylcarbamyl chloride, and β-propiolactone.
Bladder cancer

The majority of the cases of bladder cancer (BC), approximately 75%, are limited to the mucosa (stage Ta or Tis) and lamina propria (T1) at presentation, and most of these tumors can be removed by transurethral resection. Recurrence rates are high (30% to 85%), and 10% to 30% of “superficial” tumors (T1 or less) will subsequently progress to muscle invasive disease (stages T2-T4), which has a poorer prognosis (Skinner and Leiskowsky, 1988). For the remaining 25%, the initial presentation involves muscle invasive disease that will usually relapse with metastases (as well as localized disease persistence) within a median of 2 years if managed only by transurethral resection and intravesical therapy (Messing et al., 1995).

How a normal urothelial cell transforms to a malignant cell and then metastasizes is a complex process that involves the interaction of many different genes, proteins, and other molecules. Several areas of molecular research have contributed to our knowledge about the initiation and progression of BC. Loss of tumor suppressor gene function or induction of oncogenes can lead to unregulated cell growth and proliferation. Abnormal expression of growth factors, adhesion molecules, and angiogenic factors are important in the progression of BC. Due to the genomic instability of cancer cells, it has been difficult to identify those genetic, chromosomal, and transcriptional changes found in BC that are fundamental to the malignant process vs those that represent secondary or epigenetic aberrations. In general, identifying the former would be more useful in developing detection and preventive strategies, while the latter may be more valuable for prognostic purposes. Awareness of both is likely needed to develop effective therapeutic approaches.

The molecular mechanisms of BC development and progression are complicated but likely involve the interaction of tumor suppressor genes, oncogenes, growth factors, adhesion molecules, and angiogenic factors that lead a normal transitional cell to acquire the malignant phenotype. BC initiation and development involve an initial insult causing genetic derangements that often leads to either a negation of tumor suppressor genes or an induction of oncogenes. BC progression
relies on the transformed cell acquiring the properties needed to induce further growth (growth and angiogenic factors), invade through the lamina propria (cell adhesion molecules, motility factors), and establish metastatic deposits (all of these). However, further research is needed to better understand these mechanisms and pathways and thereby prevent and clinically alter the diagnosis and treatment of patients with bladder cancer.

**Autoantibodies at the intersection between cancer immunity and autoimmunity**

A common, if not intrinsic, feature of autoimmunity is a humoral immune response manifested by the production of autoantibodies targeted against self-cellular proteins and nucleic acids (Tan, 1989). Although some of the spontaneously elicited humoral immune responses observed in cancer patients recognize neo-antigens whose expression is restricted to tumor cells. Most cancer-associated autoantibodies are directed against self-antigens, such repertoire of autoantibodies overlaps to a significant extent with that typical of patients with autoimmune diseases. Autoantibodies to p53, which represent a feature of patients with systemic sclerosis, systemic lupus erythematosus (SLE) and overlap syndromes, have been found to occur also in patients with various cancer types, including lung, head and neck, breast, colon and gastric cancers (Soussi, 2000, Fernandez, 2005). Circulating autoantibodies against the c-myc nuclear protein have been detected in patients with breast, gastric, hepatic, colorectal or other types of cancer as well as in patients with SLE, mixed connective tissue disease (MCTD), dermatomyositis and autoimmune hemolytic anaemia.
Objectives of the present study

4-ABP and several other related arylamines have been shown to be causally involved in the induction of human urinary bladder cancers. 4-ABP induces a wide spectrum of tumors in a number of experimental animals. Arylamines, including 4-ABP, were earlier used in chemical industries and are present in cigarette smoke and in other sources. The formation of ABP-induced tumors in experimental animals and the detection of hemoglobin- and DNA-ABP adducts in smokers provide strong evidence for 4-ABP’s role as a carcinogen. Thus, 4-ABP and related aryl and heterocyclic amines represent an important class of environmental contaminants. 4-ABP undergoes metabolic activation by hepatic enzymes to generate the N-hydroxy metabolites, namely N-hydroxy-4-acetylaminobiphenyl (N-OH-AABP) and N-hydroxy-4-aminobiphenyl (N-OH-ABP), which are then transported to the bladder following conjugation with glucuronides. The N-hydroxyarylamines can then be converted to arylnitrenium ions through the putative intermediate N-acetoxy-4-aminobiphenyl (N-OAc-ABP) and these electrophilic nitrenium ions interact with DNA to form covalent DNA adducts, thereby exerting their genotoxic effects. The preferentially adducted sites on DNA are at the C8 position of deoxyguanosine and deoxyadenosine bases. The major (80%) DNA adduct formed has been identified as N-(deoxyguanosin-8-yl)-4-aminobiphenyl (dG-C8-ABP). Adduct levels serve as biomarkers of biologically effective dose, indicating the amount of carcinogen bound to DNA in target or surrogate tissues. In certain studies, exposure to 4-ABP resulted in a clear relationship between bladder tumor yield and DNA adduct concentration.

In the present study commercially available human DNA was modified by N-hydroxy-4-acetylaminobiphenyl. The modifications on DNA were studied by ultraviolet, fluorescence and circular dichroism spectroscopy, thermal denaturation studies, nuclease-S1 digestibility, HPLC, and LC-MS. Comet assay was performed to detect alkali labile sites, single and double stranded DNA breaks on human lymphocytes exposed N-hydroxy-4-acetylaminobiphenyl, while $^1$HNMR studies were conducted to identify the structure of the DNA-adduct formed. Antibodies against native and N-OH-AABP modified human DNA were generated in experimental
animals. The specificity of induced antibodies was evaluated by competition ELISA and gel retardation assay. The induced antibodies were also used for the detection of $N$-OH-AABP-DNA adduct in the immunohistochemical and immunofluorescence analysis of kidney sections from rabbits immunized with modified DNA. Furthermore, the antibodies were used as an immunochemical probe to detect $N$-OH-AABP induced damage in the DNA isolated from bladder cancer patients belonging to smoker and non-smoker group. Binding of autoantibodies from bladder cancer patients, in the smoker and non-smoker group, was also checked with native and $N$-OH-AABP modified DNA.