Introduction
Dilated Cardiomyopathy (DCM) is a chronic heart muscle disease characterized by impaired systolic function of left or/and right ventricle (Richardson et al., 1996). It is a major cause of severe congestive heart failure in young people and the commonest cause of transplantation worldwide. The heart is susceptible to immune mediated injury. The myocytes may be injured during or after immune response to endogenous or exogenous cardiac antigens. Immune mediated myocyte injury may be reversible and manifested as a transient depression of ventricular function and/or electrical instability, or it may be irreversible, ultimately leading to myocyte necrosis.

Mechanisms of immune mediated injury may be classified into humoral and cellular types. Humoral immunity requires recognition of an epitope by antibody molecules expressed by B-lymphocytes and depends on circulating antibodies to initiate injury. Several cardiac disorders like post myocardial infarction syndrome, idiopathic dilated cardiomyopathy, viral myocarditis and doxorubicin cardiotoxicity are accompanied by deposition of antibodies within the myocardium, particularly on the sarcolemma (Rose et al., 1988). Moreover, 13-95% patients with various myocardial diseases such as idiopathic dilated cardiomyopathy, myocarditis and hypertrophic cardiomyopathy have shown the presence of heart reactive antibodies in their serum (Neumann et al., 1990). The major mechanism of cell injury mediated by humoral immunity may be the activation of complement via the classic pathway.
Cellular immunity also plays a role in the mechanism underlying cardiac dysfunction. It is initiated by recognition of an immunogenic epitope by antigen specific T-lymphocytes. This initial recognition and activation are effected by helper T-cells and require presence of histocompatible antigen presenting cells. Presentation of myocardial antigen to T-lymphocytes by antigen presenting cells can result in the clonal expansion and differentiation of cytotoxic T-cells, stimulated by the release of immune cytokines. As a consequence, myocyte necrosis occurs, resulting in fibrosis (Sanderson et al., 1985).

Extensive studies have provided appreciable evidences for the presence of a wide spectrum of autoantibodies against cardiac tissue in myocardial diseases (Maisch et al., 1983; Caforio et al., 1992). In patients with DCM, circulating autoantibodies to distinct cardiac autoantigens have been described providing evidences for autoimmune involvement. Thus, autoimmune disorders result due to immunologic reactions, humoral and/or cellular, directed against the individuals own tissue components. The precise mechanisms underlying the trigger of autoimmune reaction are not yet completely understood, however certain factors are said to be involved.

Shoenfeld and Isenberg (1989) described the wide spectrum of autoimmune diseases as a mosaic of autoimmunity with many factors leading to diverse diseases.

**T-helper/T-suppressor cell imbalance:**

The T-cell sub-sets control / regulate the immune response. In
autoimmune diseases, the ratio of T-helper (Th) to T-suppresser (Ts) cells in peripheral blood tends to be nearly 10-15 : 1, particularly during active or acute phase of the disease compared to 2 : 1 in normal individuals (Deodhar, 1992).

**Polyclonal B-cell activation:**

Polyclonal B- and/or T-cell activation has been an initiating mechanism. The proposition that polyclonal B-cell activators can induce autoantibodies is predicted on the existence of non deleted self reactive B-cells and/or developmentally arrested anergised B-cells that might become active upon appropriate stimulation. A large number of molecules, particularly of microbial origin have been found to act as polyclonal B-cell activators and in the induction of autoantibodies. Polyclonal stimulation of a large set of T-cells by bacterial/viral surface antigens (SAgs) is another suggested scenario. T-cells that react with MHC class II bound SAg on B-cells may mutually stimulate the SAg displaying B-cells, thereby leading to production of polyclonal immunoglobulins and in some instances, autoantibodies. Alternatively, the activated T-cells themselves may induce tissue damage, through cross reactions with self molecules (Theofilopoulos, 1995).

**Genetic factors:**

Genetic transmission in Idiopathic DCM (IDC) has been occasionally described in the past while importance of genetic factors is a recent advance (Mestroni et al., 1990). In control studies,
familial IDC is detectable in over 20-25% of patients with a diagnosis of IDC, with a prevalent autosomal dominant trait. A single dominant locus was proposed and for the putative disease gene, a gene frequency in the order of $10^{-4}$ was estimated in overall population.

The candidate genes for familial IDC can be divided into two main groups, genes that are responsible for normal heart function like the ones encoding contractile proteins such as myosin light and heavy chain, tropomyosin, troponins and actin. Other candidate genes are genes coding for proteins involved in metabolic pathways: genes encoding for atrial natriuretic factor and its receptor, phospho lamban, G-proteins, β-adrenoceptors and Ca$^{2+}$ channels appear to be of particular interest. According to autoimmune pathogenetic hypothesis of IDC, the second group of genes are involved in immune function. Immune dysfunctions in IDC involves cellular and humoral immunity such as organ specific autoantibodies (Caforio et al., 1994). Association studies indicate a strong correlation between disease and human leukocyte antigens (HLA). Association between immune response (Ir or MHC) and T-cell receptor (TCR) genes and the development of immune mediated cardiac injury are not unexpected since most autoimmune diseases are T-cell dependent and all T-cell mediated responses are major histocompatibility complex (MHC) restricted (Bach, 1995). MHC may alter predisposition by shaping of TCR repertoire, antigenic peptide selection and presentation and peptide transport. HLA-DR associations play a major role in patients with DCM. HLA-DR positive patients were six times more likely to
have antibodies compared to those who did not belong to this phenotype. Antibodies against different cellular constituents may have distinct immunogenetic associations. On the other hand, HLA-DR4 and HLA-DR1 share common epitopes which may confer susceptibility to autoimmunity in DCM.

In addition, the existence of an association between autoantibodies, clinically overt disease and certain restriction fragment length polymorphisms (RFLPs) of HLA-DRβ and -DQα genes suggests that subtypes of these molecules may have enhanced pathogenetic significance (Limas, 1996).

**Immune deficiency states:**

The availability of severe combined immunodeficient (SCID) mouse provides a potentially unique opportunity to study contributions made by the immune system in induction of coxsackie virus B3 (CVB3) induced myocarditis. Unlike congenitally athymic (nu/nu) mice, the SCID mutation results in an absence of both B- and T- cell function, due to an aberrant VDJ recombinase mechanism. While no functional T- or B-cells are present in the SCID mouse, antigen presenting capabilities and natural killer cell activity are intact (Bosma and Caroll, 1991).

In 1986, Cohen et al. described the first cases of rapidly fatal DCM in three AIDS patients. Since then a number of prospective clinical and echocardiography studies have shown that a subgroup of
HIV infected patients may be predisposed to the development of clinically significant and progressive heart disease (Herskowitz et al., 1992; Herskowitz & Baughman, 1994). This subgroup of patients frequently present with class III or IV congestive heart failure and demonstrate rapid clinical deterioration. One emerging hypothesis to explain the high frequency of dilated heart muscle disease in HIV seropositive patients is the association between HIV related ventricular dysfunction and myocarditis. The increased CD8+ T-lymphocytes and sole induction of MHC class I in the HIV related myocarditis population are likely due to the marked systemic decrease in circulating CD4+ T-lymphocytes in AIDS patients (Levy et al., 1985).

Studies by Matsumori et al. (1994) have suggested alterations in cytokine production in patients with dilated heart muscle disease. Patients with HIV related myocarditis have elevated levels of TNF-α and IL-6 compared to patients without myocarditis suggesting that specific cytokines may play a role in the pathogenesis of HIV related myocarditis (Herskowitz et al., 1995).

**Molecular mimicry:**

Most studies have addressed molecular mimicry as cross reactions of antibodies with linear peptides shared by self and foreign molecules (Theofilopoulos, 1995). The term 'molecular mimicry' was initially used in 1968 to explain persistent viral infection. Autoimmunity provided by molecular mimicry should occur only when the microbial and host determinants are similar enough to cross react,
yet different enough to break immunologic tolerance. The induction and breaking of tolerance at both the B- and T-cell levels have been established in heterologous serum protein models, and the same kinetics probably govern the establishment and breaking of tolerance to microbial agents cross reacting to host proteins.

It is to be pointed out that an antibody reacts with the three dimensional configuration of a molecule, the antibody could bind but with a different affinity, to similar but not necessarily identical configurations. The multiple organ reactive antibodies recognized either the same molecule present in more than one organ or different molecules in multiple organs (Haspel et al., 1983). Molecular mimicry is a common phenomenon. The role cross reacting antibodies play in pathogenesis will depend on the nature and location of the autoantigens as well as on the properties of the antibodies (Srinivasappa et al., 1986). Homology by itself may not lead to a cross reacting immune response. However, unless the homology and subsequent immunological cross reactivity involve a host protein that can precipitate disease, the autoimmune response is unlikely to lead to autoimmune disease.

Infectious agents may participate in the induction of autoimmune disease not only through the process of mimicry, but also through other effects, including tissue damage and release of sequestered antigens, availability of cryptic self determinants through increased MHC expression, ectopic expression of molecules, redistribution of
intracellular molecules to the cell surface, upregulation or shift in the spectrum of cytokine production, immunological exhaustion and bystander activation of T-cells (Theofilopoulos, 1995).

In humans, myocarditis is known to be the precursor of DCM in some cases, although clinical and histological diagnosis remains obscure (Aretz et al., 1985). The commonest cause of myocarditis is said to be a virus infection, but majority of cases are of unknown etiology (Woodruff, 1980). Pedigrees where myocarditis and DCM were present in different family members have been reported (O'Connell et al., 1983).

Since in DCM, the only affected organ is the heart muscle, to prove autoimmune involvement, it is necessary that the criteria of an organ specific autoimmune disease are fulfilled (Rose and Bona, 1991). Autoimmune features in DCM include familial aggregation, a weak association with HLA-DR4 and immunoglobulin genes, abnormal expression of HLA class II on cardiac endothelium and increased levels of circulating cytokines. The organ-specific antibodies produced a diffuse cytoplasmic staining of myocytes but did not stain skeletal muscle. Antibodies were classified as cross reactive 1, which exhibit only partial heart specificity, gave a fine striational staining on cardiac tissue and stained weakly skeletal muscle fibres. The entirely cross reactive antibodies, classified as cross reactive 2, stained with a broad striational pattern both in heart and skeletal muscle sections. Absorption studies with relevant tissues had confirmed the organ
specificity and cross reactivity of the antibody types (Caforio et al., 1990).

Like other autoimmune conditions, DCM autoantibodies seem to be produced against multiple antigen specificities. The multiplicity of parallel autoimmune reactions directed towards a single organ could be due to spread sensitization. Thus, a single, initial autoantigen would give rise to the first attack of the target organ, which would result in T-cell mediated inflammation or the release of degradation products. These mechanisms would enhance autoantigen presentation or release immunogenic cell components that would give rise to a secondary autoimmune response that would be difficult to differentiate from the primary one. Conversely, as suggested, the initial event is a target cell abnormality or lesion that renders a set of immunogenic peptides: only the peptide with a sufficient affinity for MHC molecules would be recognized by the T-cell repertoire, leading to an autoimmune response (Bach, 1995).

Several autoantigens which react with sera from patients with DCM have been identified. Studies on tissue from patients of myocarditis and DCM have identified increased expression of intracellular antigens such as adenine nucleotide translocator (ANT) and branched chain α-ketoacid dehydrogenase (BCKD) complex. Antibodies against the intracellular mitochondrial ANT protein cross react with myocyte sarcolemmal calcium ion (Ca^{2+}) channel proteins. Binding of Ca^{2+} channel proteins by such cross reactive antibodies can
thus physiologically alter the metabolism of normal myocyte, possibly leading to myocyte injury (Ansari et al., 1991). Since SR-Ca$^{2+}$ ATPase constitutes the major calcium regulatory protein in the sarcoplasmic reticulum regulating myocardial contractility, autoimmune inhibition of this enzyme may affect the functional outcome of the myocardium. The time dependent association between SR-Ca$^{2+}$ ATPase immunization and development of severity of myocarditic lesions supports the immunopathogenetic role of SR-Ca$^{2+}$ ATPase. The involvement of T-cell immunity may contribute more to the generation of myonecrosis, whereas the antibody mediated immunity may contribute more to functional impairment and activation of complement (Sharaf et al., 1994). The ADP/ATP carrier has organ specific antigenic determinants although there is a partial identity among the carrier proteins from heart, kidney and liver. The organ specificity of ADP/ATP carrier was also confirmed by the organ specific reaction of dilated cardiomyopathy sera with this protein. The autoantibodies reacted with antigenic determinants at the cardiac myocyte plasma membrane and interacted with at least one subunit of calcium channel. Therefore, the autoantibody was also called the Ca-channel reactive antibody. A cross reaction between antigenic determinants could be of pathogenic significance because antibodies against the homologies were present in patients with myocarditis and DCM (Ansari et al., 1988; Schwimmbeck et al., 1993).

After an autoimmune response is initiated, circulating autoantibodies against the ADP/ATP carrier disturb myocardial energy
metabolism and cross react with myocyte sarcolemmal Ca\(^{2+}\) channel proteins. The antibodies were able to bind to the Ca\(^{2+}\) channel and enhance calcium influx and calcium overload in cardiac myocytes, finally resulting in cytotoxic damage (Liao, 1996).

The most important intracellular microorganisms associated with viral disease include a variety of viruses (Woodruff, 1980), two members of chlamydiae (*Chlamydia psittaci* and *Chlamydia pneumoniae*) (Kuo et al., 1993), several rickettsiae, *Toxoplasma gondii* and *Toxoplasma cruzi* (Speirs et al., 1988). *Trichinella spiralis* is the most prone worm to cause myocarditis. Myocarditis pathogenesis may be complex and involve various immune mechanisms. In addition, many gram-positive and gram-negative bacteria may persist and multiply intracellularly or extracellularly or both and cause damage to the myocardium by various pathways. Some bacteria produce exotoxins that may cause damage to the myocardium, whereas others release endotoxins that may be harmful. The bacteria commonly encountered in myocarditis are Beta-hemolytic streptococci (Putterman et al., 1991), *Corynebacterium diptheriae* (Havaldar, 1992), *Neisseria meningitidis* (Hardman and Earle., 1969), *Yersinia enterocolitica* (Zollner et al., 1992), *Salmonella typhi* or *paratyphi* and *Borrelia burgdorferi* (Klein et al., 1991). Mycoplasma pneumoniae was incriminated in 6% of consecutive military conscripts suffering from unequivocal myocarditis, and in 1-2% of conscripts hospitalized with mycoplasmal infections (Karjalainen, 1990). *Trypanosoma cruzi* (Chagas' disease) is a well recognized cause of myocarditis and
cardiomyopathy in both urban and rural areas of South America. The African trypanosomes, *T. gambiense* and *T. rhodesiense* occasionally cause myocarditis and cardiomyopathy (Tsala Mbala, 1988).

Coxsackie virion and other viral proteins share epitopes with internal or plasma membrane proteins of normal cells (molecular mimicry) and stimulate immune responses which participate in autoimmune reactions. These hypotheses may not be exclusive and all may be operative in a single model. CVB3 or CVB4 particles share epitopes with human cardiac myocyte sarcolemmal proteins, human and mouse cardiac myosins, streptococcal M-protein, adenine-translocator protein and an unidentified protein(s) on the plasma membrane on normal mouse cardiac myocytes or fibroblasts (Srinivasappa et al., 1986).

This underlines the clearence of virus via rapid and other immune responses significantly affects the outcome (acute resolved versus acute/chronic myocarditis). Antecedent infections by heterologous CVB3 serotypes can exacerbate or ameliorate in CVB3 induced myocarditic challenge mechanism by which shared epitopes between viral cellular proteins can provide either protection or increase severity of CVB3 induced myocarditis. Studies by Gauntt et al. (1995) suggest that antibodies to only a limited numbers of epitopes on the light meromyosin (LMM) rod portion of human cardiac myosin (HM) are shared with CVB3m and participated in protection/enhanced disease and antibodies to the majority of shared epitopes in LMM are
without consequence in CVB3m induced heart disease. Resistance to this enterovirus and other intracellular infections partly depends on natural killer cells (NK) which are vulnerable to the effects of environmental pollutants such as mercury, nickel, 8-tetrachlorodibenzo-p-dioxin and methyl mercury. The development of disease as seen in the heart of CB3 virus infected mice, can be severely affected in different ways by different heavy metals. Furthermore, during infection, the organ distribution of heavy metals may be changed in a specific way for each compound. This may subsequently result in altered infectious disease pathogenesis (Ilback et al., 1995).

The G-protein coupled membrane receptors are all members of a superfamily of membrane proteins which structurally share the overall bacteriorhodopsin architecture, seven α-helices, spanning the membrane lipid bilayer and forming a hydrophobic pocket that can serve as a pharmacophore. The seven helices are linked together by three extracellular and three intracellular loops. The N-terminus of the polypeptide is located in the extracellular space while the C-terminus is located in the intracellular space. In order to induce an immune response against G-protein coupled receptors, two requirements are necessary: firstly the receptor must be degraded by proteolysis to small fragments and the fragments generated must be able to form a complex with one of the MHC or HLA class II molecules of the host.

The functionally important G-protein coupled receptors are β-adrenoceptor and the M2 muscarinic receptor. Anti-β receptor
antibodies were present in 30-40% patients of dilated cardiomyopathy. These antibodies were produced against $\beta_1$ peptide corresponding to the second extracellular loop with little reactivity towards a $\beta_2$ peptide in patients with DCM. $\beta_1$-adrenergic receptor has two antigenic regions. Some of the sera recognize the first extracellular loop, other sera the second extracellular loop of the $\beta_1$-adrenoceptor. The dominant epitope in the second loop is the cysteine-containing amino acid sequence A-R-R-C-Y-N-D which forms a disulphide bridge with the cysteine of first extracellular loop (Wallukat et al., 1995). The immunoglobulin fraction isolated from serum samples of myocarditic and cardiomyopathic patients contains stimulatory autoantibodies, directed against $\beta_1$-adrenoceptor which increase dose dependently, the beating rate of the cultured neonatal heart myocytes. Recent studies have pointed out the presence of anti-receptor autoantibodies in DCM (Fu et al., 1994). Immunization of genetically predisposed mice with cardiac myosin causes cardiac enlargement, the production of heart-specific antibodies and a heart condition that resembles human DCM. the autoantibodies in patients against human ventricular myosin are directed against myosin heavy chain. The $\alpha$- and $\beta$-myosin heavy chains are relevant autoantigens by the heart specific antibodies detected by immunofluorescence in DCM. In humans, the $\alpha$-myosin heavy chain isoform is expressed exclusively in atrial myocytes, whereas the $\beta$-isoform is present both in ventricular myocytes and slow skeletal muscle fibers (Caforio et al., 1992). Cardiac myosin induced myocarditis is T-cell mediated and class II MHC restricted.
Myosin-reactive T-cells from normal myosin immunized donors induced severe myocarditis in severe combined immunodeficiency (SCID) mice. Potential myosin reactive T-cells, though lacking CD4 or CD8, still remain functional and hence CD4 or CD8 molecules are not essential for induction of autoimmune myocarditis (Pummerer et al., 1995).

Myosin is an intracellular molecule. Viral infection or other causes of tissue necrosis might lead to release or exposure of myosin and trigger autoimmunity in individuals with a predisposing genetic background. Virus may promote auto-sensitization to myosin by molecular mimicry (Oldstone, 1987; Srinivasappa, 1986). Monoclonal antibody to coxsackie VP-1 capsid protein has been demonstrated which reacts with cardiac myosin heavy chain.

The mechanism by which myosin as well as other autoantigens previously identified may trigger and perpetuate, although not directly cause autoimmune disease is unknown. It also remains to be established whether the antibodies found in DCM sera have a direct pathogenic role or, like the anti-myosin antibodies found in murine autoimmune myocarditis, are only markers of immune damage.

**Role of 150 kD cardiac C-protein:**

Cardiac C-protein is found to be a dominant antigen among many types of cardiac proteins and is known to induce autoimmune myocarditis as judged on the basis of experimental studies indicating
production of autoantibody by mouse specifically against 150 kD C-protein on induction of myocarditis. This specific antibody is produced inspite of immunization with whole cardiac tissue homogenate. Purified C-protein also produced myocarditis and recombinant cardiac C-protein (amino acid residue 205-916) effectively produced myocarditis in some mice strains including SMA, DBA/1J, SJL, and 02°/A, but not in A/J, AKR/J, or DBA/2J mice strains (Kasahara et al., 1994). C-protein is a component of thick filament of skeletal and cardiac muscles (Yamamoto and Moos, 1983). The function of C-protein remains unknown, but it has been suggested that C-protein may regulate thick filament assembly and length (Squire, 1981), participate in thick filament structural support (Pepe et al., 1975), maintain the structure and contribute to the radial elasticity of the sarcomere, or regulate cross-bridge movement during contraction (Magid et al., 1984).

C-protein has a number of properties that make particularly attractive the suggestion that it plays a role in regulation of contractile activity. For example, C-protein binds to both purified actin (Moos et al., 1978) and myosin (Moos et al., 1975) and can alter the ability of actin to stimulate myosin ATPase (Moos and Feng, 1980). The effects of C-protein on ATPase activity are, however, complex. C-protein inhibits skeletal muscle actomyosin ATPase, but stimulate cardiac muscle actomyosin ATPase (Yamamoto and Moos, 1983). It has been suggested that the physiological role of C-protein may involve a calcium-regulated binding of C-protein to thin filaments, because C-
protein binding to native thin filaments occurs only in the presence of micromolar concentrations of calcium (Moos, 1981). It has been shown that C-protein in cardiac muscle becomes phosphorylated in response to β-adrenergic agonists and dephosphorylated in response to cholinergic agonists (Hartzell and Titus, 1982). The level of protein phosphorylation correlates with the rate of relaxation of cardiac contraction and it has been suggested that C-protein regulates twitch relaxation in cardiac muscle. Electron microscopy shows C-protein to be V-shaped. The other particles could be seen as distorted, stretched or collapsed V-shaped particles.

C-protein is one of the major constituent protein necessary to compete with natural antigens such as invariant chain and MHC to obtain the position at the MHC-antigen binding site (Demotz et al., 1990; Harding and Unanue, 1990). Moreover, C-protein is an intracellular member of the immunoglobulin superfamily (Einheber and Fischman, 1990). IgG like domains are conserved in most cell surface immune recognition molecules and also TCR and MHC class II have this domain (Williams and Barclay, 1988). The peptides of recycled membrane proteins, class II HLA and invariant chain, have been shown to be major self-antigens which are presented by class II molecules. However, the HLA binding motifs are located in the variable domain of this structure, not in this constant IgG like domain (Rudensky et al., 1992; Baum et al., 1993). The question remains whether or not these IgG like conformations work with each other at cell surface similar to the directly bound MHC-TCR like superantigen (Woodland and Blackman, 1993).
The findings of Kasahara et al. (1994) conflict with an earlier report that myosin is the major antigen producing autoimmune myocarditis under H-2 genetic restriction (Neu et al., 1987). In SMA mice, repeated injections of myosin-enriched heart extract with KO3 LPS still preferentially produced the autoantibody to C-protein. In addition, myosin contamination was completely avoided by using the fusion protein expressed in *E. coli* P16-4, which is encoded by part of the cDNA of C-protein. CB3 induced myocarditis which might further trigger autoimmune reactions against myosin by a molecular mimicry mechanism, has been suggested to be a possible underlying pathogenesis in DCM (Noel et al., 1991). Kasahara et al. (1994) suggested that the autoimmune triggering mechanism in C-protein induced myocarditis may differ from a CB3-triggered or myosin-triggered mechanism because cross-reactive epitopes between CB3 and C-protein have not been reported; contrary to the previous results that A/J, C3H/He, Balb/c, DBA/2 strains have a high susceptibility to CB3 (Lawrence et al., 1991), the C-protein residue 205-916 hardly induced myocarditis in these strains.

Further assessment about the involvement of C-protein triggered autoimmune myocarditis in DCM, immunoblotting was employed with sera of 16 DCM patients against whole cardiac proteins. Out of 16 DCM patients sera of 2 patients preferentially recognized the C-protein. On the contrary, none of the control sera reacted specifically to C-protein (Kasahara et al., 1994).
crossreactivity. The maximum score possible for each peptide is variable and is determined by its length and by the amino acids present, rarer amino acids having a higher value. The scoring system used, attaches the highest values to exact matching of rare amino acids. This level of matching would be expected to lead to cross reactivity of epitopes since far weaker matching occurs within conserved motifs of peptides bound to class II major histocompatibility complex peptides. Differences between individuals in MHC class II may influence the selection of particular hsp epitope and the corresponding target antigen that gives rise to an autoimmune disease (Jones et al., 1991).

The amino acid sequences/peptides of autoantigen region 384-396 and 451-470 of hsp share homology with the amino acid sequences/peptides of myosin heavy chain 136-148 and 253-272 with a similarity score of 65 and 64, respectively, as seen in coxsackie myocarditis (Jones et al., 1991). Latif et al. (1993) have reported antibodies against a 60 kD band in 85% DCM patients by Western blotting. Two dimensional gel analysis and protein sequencing revealed 100% homology of this peptide with hsp 60. The presence of circulating antibodies against hsp 60 in 85% of DCM patients is clear evidence of a damaged myocardium. This suggests that myocyte injury induces the expression of hsp60 or other stress proteins that may have immunomodulatory activities and could influence the development and course of autoimmune recognition of the myocardium. The exact pattern and distribution of hsp60 in normal and diseased myocardium remains to be established.
DNA-protein photoadducts/photointeractions:

A variety of chemicals and ionizing and ultraviolet (UV) radiations are known to produce DNA-protein crosslinks (Oleinick et al., 1986). Several reports indicate that the bonds formed between DNA and proteins are of covalent nature (Oleinick et al., 1986; Cress and Bowden, 1983). Proteins and nucleic acids are the two most important classes of functional biomolecules in cells. These "colourless" macromolecules absorb in the UV region of spectrum. Typical photochemical reactions have been identified that are relevant to biological damage induced by exposure of organisms to radiation.

The crosslinking of nucleic acids to protein is one of the lesions produced in biological systems by UV light, which has been observed in bacteria (Smith, 1962; Alexander and Moroson, 1962; Bridges et al., 1967) and in mammalian cells (Alexander and Moroson, 1962; Habazin and Han, 1970; Han et al., 1975; Zimmernan et al., 1972). The photobinding of serum albumin to DNA (Maukovitz, 1972) and gene-5-protein to bacteriophage T4DNA (Anderson et al., 1975) provides direct proof at a molecular level for the crosslinking phenomenon. The role of DNA-protein crosslinks in aging, carcinogenesis and radiation biology has been well documented (Smith, 1975).

The recognition between nucleic acids and proteins is of fundamental importance since it triggers most steps of nucleic acid metabolism. This recognition, presumably, first involves electrostatic
interactions of phosphate groups with positively charged regions in proteins; then, more specific interactions take place which involve possible interactions between nucleic acids bases (or other structural elements) and amino acids side chains in proteins. A more extended scope of examination reveals the possible recognition of secondary or tertiary structures of nucleic acids by proteins. In either cases, recognition may be dependent of nucleotide sequence (Duguet, 1981).

The initial isolation of a mixed photoproduct of thymine and cysteine (5-S-cysteine-6-hydrothymine) from in vitro UV irradiation of a solution of thymine and cysteine has served as a model for crosslinking phenomenon. Fifteen amino acids have been found to react photochemically with DNA. Cysteine, lysine, phenylalanine, tryptophan and tyrosine were the most reactive; alanine, aspartic acid, glutamic acid, serine and threonine are unreactive (Shetlar et al., 1985). It has been suggested that the resistance of *Micrococcus radiodurans*, one of the most radiation resistant organisms known, is due to its extraordinary ability to repair pyrimidine dimers, but what ultimately kills the organism is damage that involves both DNA and protein. The crosslinking of DNA and protein may constitute one type of such damage (Shetlar, 1980).

A different type of photochemistry arises when protein and DNA are irradiated together as compared to when they are irradiated separately. Since, DNA and proteins do not exist in cells as pure solutions of the separate molecules but are in intimate contact with
each other, it is suggested that photochemical interaction of DNA and protein would play a significant role in the inactivation of UV irradiated cells under certain conditions.

Hydroxyl (OH) radicals produced from water by ionizing radiation appeared to be responsible for the formation of ionizing radiation induced DNA-protein crosslinks in isolated chromatin and in intact cells (Oleinick et al., 1986; Mee and Adelstein, 1981). The nucleosomal core of the histones as well as non-histone proteins has been found to be involved in the formation of DNA-protein crosslinks in isolated chromatin exposed to ionizing radiation (Mee and Adelstein, 1981). The hydroxyl radical induced formation of DNA-protein crosslink between methyl group of a Thy moiety and the C-3 position of a Tyr moiety in calf thymus nucleohistone in aqueous solution has been described (Dizdaroglu et al., 1989).

DNA-protein crosslinking in normal and solar UV sensitive ICR 2A frog cell lines exposed to solar UV radiation has been observed (Rosenstein et al., 1989). Induction of DNA-protein crosslinks in these mutant cell lines may help in examining the role played by this type of DNA damage in the hypersensitivity to solar UV radiation which are exhibited by these mutant cells. These crosslinks might be lethal to the cell if they interfere with normal gene functions.

Role of free radicals:

A free radical can be defined as a chemical species possessing an unpaired electron and is capable of independent existence. Free
radicals can be formed by:

i) Homolytic cleavage of a covalent bond of a normal molecule, with each fragment containing one of the unpaired electrons

ii) By the loss of a single electron from a normal molecule

iii) By the addition of a single electron to a normal molecule.

The prima donna in the biochemistry of oxygen free radicals are oxygen itself, superoxide, hydrogen peroxide, transition metal ions and the hydroxyl radical, the first four of which conspire by a variety of interactions to generate the last (Halliwell and Gutteridge, 1990). Under normal circumstances, the major source of free radicals in cells is electron 'leakage' from electron transport chains. These can be mediated by the action of enzymes or non-enzymatically, often through the red-ox chemistry of transition metal ions (Cheeseman and Slater, 1993). Free radical production can also be increased by exogenous sources like toxic foreign compounds and ionizing radiations. Although there are several sites of ROS generation in cells, the main source of O₂⁻ and its stoichiometric product, H₂O₂ is the auto oxidation of the reduced components of the mitochondrial electron transport chain (Haiku et al, 1993). In the presence of trace amounts of transition metal (M) ions (i.e. Fe²⁺, Cu⁺⁺) found in biological systems (Halliwell and Gutteridge, 1985), H₂O₂ can participate readily in Fenton-like reactions (Fenton, 1894; Cohen, 1985) resulting in the production of ·OH as shown.
\[
\text{Mn}^+ + \text{H}_2\text{O}_2 \rightarrow \text{M}^{(n+1)} + \cdot\text{OH} + \cdot\text{OH}
\]

Hydroxyl radicals have been implicated in the deleterious processes such as gene mutation (Okada, 1970), cell transformation (Borek, 1985) and cell death (Painter, 1980). Nitric oxide (NO') is an important physiological free radical produced by phagocytes in which the unpaired electron is delocalized between two atoms. Nitric oxide or its derivative acts upon smooth muscle cells in vessel walls to produce relaxation, besides acting as a neurotransmitter (Hirono et al., 1997).

**DNA damage by ROS:**

DNA is an important target for free radicals since it is clearly of major significance for cell function as well as its susceptibility of being damaged by oxidizing radicals (Wiseman and Halliwell, 1996). The most important reactions seems to be based on hydrogen abstraction to form carbon-centered radicals followed by oxygen addition to form peroxyl radicals that subsequently decay. Addition of \cdot\text{OH} to double bonds is also an important mechanism. Both the nucleobases and the sugar phosphates offer potential targets for hydroxyl radicals (\cdot\text{OH}). The consequences of oxidative radical damage to DNA are highly serious which includes cell death, reproductive cell death, mutation and transformation into cancer cells (Feig et al., 1994). Oxidative damage to DNA includes a range of specifically oxidized purines and pyrimidines as well as alkali labile sites and strand breaks formed directly or by repair processes.
The apparently most abundant and certainly the most studied oxidative modification of DNA bases involves the C-8 hydroxylation of guanine, frequently estimated as the oxidized deoxynucleoside, 8-oxo-7,8-dihydro-2'deoxyguanosine (8-oxodG). Oxidation of guanine can also lead to the ring opened product of 2,6-diamino-4-hydroxy-5-formamido pyrimidine (Dizdaroglu, 1991). Other abundant oxidatively modified purines and pyrimidines include 8-oxoadenine, 2-hydroxyadenine, Fapy adenine, 5-hydroxy methyluracil, 5-hydroxycytosine, cytosine glycol and thymine glycol (Tg). In addition, a large number of other modifications of bases and sugars have been identified (Dizdaroglu, 1991; Dizdaroglu, 1994). All these modifications are present in DNA, and the level can be increased in vivo or in vitro by systems generating ROS. The yield of the individual DNA modifications is highly dependent on which ROS are involved. Thus, whereas, singlet oxygen induces preferentially 8-oxodG (Epe, 1991), superoxide has low reactivity to induce this modification at all. Apriori to the above, hydroxyl radicals can cause almost any modification (Dizdaroglu, 1991). In addition, the target molecule conditions, such as oxygen tension, chelation of transition metals and the presence of reductants may influence the yield and nature of oxidative DNA modifications (Dizdaroglu, 1994).

Oxidative modifications of DNA bases can lead to mutations if left unpaired or repaired with errors before replication. From studies on bacteriophage and plasmid DNA, it appears that although radicals
generated by ionizing radiations damage all four bases, mutations are usually related to modifications of GC base pairs in contrast to AT base pairs. Thus, AT base pair perturbations/modifications rarely lead to mutations. The mutations are clustered in hotspots and are mainly base pair substitutions, whereas, base deletions, large deletions, and insertions are less frequent. Hydroxyl radicals generated from ionizing radiation induce mainly GC to CG or AT base pair substitutions, depending on DNA expression system, whereas, hydrogen radicals preferentially induce GC to AT substitutions (Retel et al., 1993). Singlet oxygen and 1,2-dioxetanes preferentially induce 8-oxodG and GC to AT base pair substitutions (Epe, 1991; Retel et al., 1993; Emmert et al., 1995). In agreement, modified guanine such as 8-oxoguanine and apurinic sites are mispaired mainly with adenine, at a frequency dependent on the adjacent base sequences and the involved polymerase (Kamiya et al., 1995). Majority of ROS induced mutations appear to involve modification of guanine, in particular 8-oxoguanine, causing G to T transversions. In addition to the mutations related to mispairing to oxidised bases, ROS may also cause reduced fidelity of DNA β polymerase (Feig and Loeb, 1993) and alter the methylation of cytosine and thus gene control (Weitzman et al., 1994; Loft and Poulsen, 1996).

**Protein damage by ROS:**

Most proteins are susceptible to modification by ·OH or by ·OH + O$_2^-$( + O$_2$). The modification includes altered molecular weight
(aggregation or fragmentation), altered net electrical charge (+ or -),
loss of tryptophan, and production of bityrosine. Since \( \cdot \text{OH} \) alone has
no measurable effect on any of these parameters, it has been suggested
that \( \cdot \text{OH} \) is the initiating species. Oxygen and/or \( \cdot \text{O}_2 \) appear to modify
significantly the damage induced by \( \cdot \text{OH} \). Protein fragmentation has
been reported following exposure to \( \cdot \text{OH} + \cdot \text{O}_2 \) (Garrison et al.,
1962; Schuessler and Schilling, 1984). This process involves hydrogen
abstraction by \( \cdot \text{OH} \) from amino acid \( \alpha \)-carbon atoms, followed by
reaction with \( \text{O}_2 \) to produce peroxyl species. Decomposition of carbon
peroxides was proposed as the mechanism for protein fragmentation
(Garrison et al., 1962).

All amino acids in the protein are susceptible to modification
by \( \cdot \text{OH} \) or by \( \cdot \text{OH} + \cdot \text{O}_2 \). In contrast, \( \cdot \text{O}_2 \) alone appeared only to
reduce cysteine residues. In agreement with known rate constants for
reaction of free amino acids with \( \cdot \text{H} \), tryptophan, histidine and cysteine
are more vulnerable than most other residues. Tyrosine is formed as a
simple addition product of \( \cdot \text{OH} + \text{phenylalanine} \). The selectivity of
\( \cdot \text{OH} \) for tryptophan, tyrosine, histidine and cysteine is less than might
expected from reactions with free amino acids (Dorfman and Adams,
1973). The process of aggregation by \( \cdot \text{OH} \) appears to involve
intermolecular bityrosine formation. It is highly unlikely, however,
that bityrosine is the only (non-disulfide) covalent modification.
Essentially any amino acid radical formed within a peptide chain could
crosslink with an amino acid radical in another protein. Alternatively,
some radicals may crosslink with amino acid molecules. Protein
denaturation also preceded increases in proteolytic susceptibility following exposure to \( \cdot \text{OH} + \text{O}_2 \). Thus denaturation/increased hydrophobicity is a possible cause for enhanced proteolysis. Oxidatively denatured proteins are degraded by novel ATP and calcium independent, soluble proteolytic systems (Davies et al., 1987).

**ROS in SLE:**

Antibodies to DNA in SLE have been extensively studied and may be specific for ssDNA, reactive with both ssDNA and dsDNA or specific for dsDNA (Worrall et al., 1990; Stollar, 1979). However, it is antibodies to native dsDNA that represent a characteristic serological finding in these patients and are of important diagnostic and clinical value. It has been shown that modification of DNA can render it more immunogenic (Ali et al., 1985). Native dsDNA has been reported to be a weak immunogen (Tan and Stoughton, 1969) and yet in certain autoimmune conditions like SLE, autoantibodies to multiple nuclear antigens, including DNA and histones are formed. Blount et al. (1991) postulated that DNA damaged by ROS may affect the development of autoimmune diseases, like SLE. The process by which DNA is damaged in such cases may involve the release of ROS intermediates during the characteristic respiratory burst of activated neutrophils, denature DNA in such a way that SLE serum anti-DNA antibodies bind better to it (Blount et al., 1989). ROS denaturation exposes base residues in the DNA backbone and minor regions of ssDNA. Lymphocytes isolated from patients with SLE contain
increased levels of 8-oxodG in the DNA. In urine, the SLE patients did not secrete 8-oxodG. A study on cultivated blood monocytes isolated from SLE patients showed reduced capacity for repair of 8-oxodG induced by incubation with hydrogen peroxide. In DNA isolated from immune complexes precipitated from plasma, 8-oxodg was a 100 fold more abundant in SLE patients compared to usual values of nuclear DNA. This oxidatively modified DNA may be a source of DNA antibodies characteristic for SLE. The SLE patients suffer from an increased rate of oxidative DNA damage and also from deficient repair. This may contribute to the pathogenesis and even to increased risk of malignant diseases in these patients (Lunec et al., 1994; Bashir et al., 1993). Hydroxyl free-radical mediated in vitro modification of calf thymus DNA showed single strand breaks, decrease in melting temperature and structural alteration of purine and pyrimidine bases leading to polyspecificity of induced antibodies (Alam et al., 1993, Ara and Ali, 1993). Antibodies against free radical modified DNA recognize B-conformation (Ara et al., 1992). ROS modified DNA promises to be a more discriminating antigen than native DNA for binding of SLE autoantibodies (Ara and Ali, 1993) and the binding increased considerably with increase in DNA fragment size (Ara and Ali, 1992).

**ROS/RNS in autoimmune myocarditis/DCM:**

Oxygen derived free radicals and their metabolites have been implicated to participate in various types of myocardial injury. These
radicals and metabolites are thought to mainly consist of superoxide
anion (O$_2^-$) hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (·OH),
however, the culprit playing the most important role is still
undetermined. The involvement of H$_2$O$_2$ generated through dismutation
of O$_2^-$ caused severe myocardial damage (Kloner et al., 1989). Oxygen
derived free radicals and their metabolites can cause enzyme
inactivation either directly or indirectly (Miki et al., 1988). Thus,
functional deterioration may be linked to ATP reduction by inactivation
of enzymes necessary for glycosylation and oxidative phosphorylation
(Goldhaker et al., 1989). On the other hand, calcium transport in the
sarcoplasmic reticulum has been demonstrated to be decreased by
exposure to oxygen radicals (Hess et al., 1984). ·OH, on the other
hand, is produced by H$_2$O$_2$ through iron catalysed Haber Weiss reaction
(Haber and Weiss, 1934) and is highly reactive. It causes lipid
peroxidation of the biological membranes (Meerson et al., 1982)
resulting in changes in membrane permeability and fluidity, and finally
results in loss of membrane integrity. The plasma membrane and
mitochondria are more damaged in the heart exposed to higher ·OH
levels. The combination of these changes may have resulted in
decreased contractility in hearts exposed to ·OH (Takemura et al.,
1994). Experimental studies by Fukuchi et al. (1991) have indicated
the possible involvement of free radicals and antioxidants in the early
stages of the development of cardiomyopathy in BIO 14.6 Syrian
hamster.
Recent studies on the molecular genetics of the myocardium have revealed that a certain percentage of patients with DCM possess mutation in the mitochondrial (mt) DNA of the myocardium. These patients can be regarded as microchondrial cardiomyopathy (mtCM), a new clinical entity. The cumulative increase in oxygen free radical damage in human heart mt DNA closely correlates with deletions associated with age and with mtCM. This led us to the 'redox mechanism of ageing and degenerative disease' in which the oxygen free radical damage in mt DNA results in a cumulative increase in somatic mutations, leading to bioenergetic deficit, cell death, ageing and degenerative diseases. The accumulation of hydroxyl radical adducts in mt DNA hydrolysate, such as 8-hydroxydeoxyguanosine (8-OH-dG) in place of deoxyguanosine (dG), could satisfy this prerequisite to the deletion (Hayakawa et al., 1992). The mt DNA, having no protective protein, is located inside the inner mt membrane. During the evolution from yeast to mammals, mt DNA reduced to one fifth of its size by loss of introns. Thus, human mt DNA more easily acquires oxygen free radical damage than single celled organisms. The revelation that human mt DNA is highly susceptible to hydroxyl attack resulting in breakdown into hundreds of mini circles suggests that the major target of the active cell death machinery would be mt DNA. The cumulative and synergistic increase in oxygen damage and deletions in mt DNA associated with age implies that normal ageing and development seem to be the two sides of a coin. The cell death machinery could be easily imbalanced by the point mutations,
especially (syn'), in the mt DNA of patients with mtCM, leading to their premature ageing (Ozawa, 1995).

In recent years, nitric oxide (NO) has been implicated in a wide variety of immunologically mediated disorders. Pathologic release of NO is considered to be one of the probable cause of diminished myocardial contractility such as septic shock (Brady et al., 1992), cardiac allograft rejection (Yang et al., 1994) and some form of idiopathic dilated cardiomyopathy (Winlaw et al., 1994). Human myocarditis varies greatly in origin and is generally a self limiting process; acute congestive heart failure may develop during the active phase, but cardiac function improves significantly in most cases as myocarditis resolves histologically (Mason et al., 1995). It is possible that the excessive production of NO by inducible nitric oxide synthase (iNOS) is responsible for the development of myocardial organic lesions as well as for functional changes in myocardial contractility during the active phase of the disease.

Physiological actions of NO are mediated through the activation of soluble guanylate cyclase and the consequent elevation of intracellular cGMP level (Moncada and Higg, 1993). Studies suggest that NO modulates myocardial contractility through an increase in myocardial cGMP. It is reported that cGMP produces inhibition of Ca\(^{2+}\) influx into myocytes (Levi et al., 1989) or attenuation of the positive inotropic effect of β-adrenergic stimulation (Watanabe and Besch Jr., 1975). It is also possible that excessive and prolonged
production of NO by iNOS in infiltrating inflammatory cells modulates the mitochondrial respiration in surrounding viable myocytes, thus NO synthase inhibition in the early phase of viral myocarditis may alter the adequate immune response to invading cardiotropic virus, thus preventing the elimination of virus from cardiac tissue (Hirono et al., 1997).

**Systemic lupus erythematosus:**

Systemic lupus erythematosus (SLE) is a multisystem prototype autoimmune disease (Isenberg and Shoenfeld, 1987) which is characterized by the appearance of circulating of autoantibodies that bind to numerous cellular and macromolecular antigens of self and exogenous origin. Although there is a distinct correlation between SLE and immune system, SLE continues to remain as a disease of unknown etiology and origin. It has been postulated that immune responses against host antigens could result from genetic predisposition, exaggerated B-cell activity, cross reactivity between foreign and host antigens or modification of host antigen as a consequence of infection, inflammation, drug administration etc.

A lot of literature is available on the production and assay of antibodies to DNA, structural analysis of specific antibody-nucleic acid interaction and their application in biomedical research (Stollar, 1992). A central paradox of the pathogenesis of SLE is the origin of anti-DNA antibodies. DNA to which most of the antibodies are detected in SLE and other autoimmune disorders, is no longer regarded
as the inciting antigen because immunization with native DNA does not induce SLE like disease. Animals immunized with covalently/non covalently modified DNA induced high titre antibodies, which are almost exclusively directed towards modified structures (Stollar, 1989). Genes encoding anti-DNA antibodies, however, are not restricted to SLE and are part of normal B-cell repertoire (Cairns et al., 1989; Stewart et al., 1993). The possible role of immunogenic DNA, DNA-psoralen crosslink, hydroxyl radical, positively charged amino acids and β-estradiol in the pathogenesis of SLE has also been reported (Pisetsky et al., 1990; Hasan et al., 1991; Moinuddin and Ali, 1994; Alam et al., 1993; Ara and Ali, 1993; Ara and Ali, 1992; Alam et al., 1992).

Anti-DNA antibodies have been shown to cross react with polynucleotides (Lafer et al., 1981), polynegatively charged structures such as phospholipids (Smeenk et al., 1987), proteoglycans (Faaber et al., 1986), cardiolipin (Eilat et al., 1986), dextran sulphate (Wollina, 1985), membrane associated proteins (Jacob et al., 1984), intermediate filament of cytoskeleton (Andre - Schwartz et al., 1984), histones (Gohill and Fritzler, 1987), bacterial products (Carroll et al., 1985), platelets (Zouali et al., 1988), hyaluronic acid and chondroitin sulphate (Faaber et al., 1984). Monoclonal anti-DNA antibodies of both human and murine origin showed cross reactivity with antigens other than DNA (Shoenfeld et al., 1983). There are many compounds in the vicinity of DNA which may react upon irradiation. These compounds include polyamines, histones and nuclear matrix elements. Polybasic
molecules found in cells have great affinity for acidic constituents such as DNA (Held and Awad, 1991) and their interaction may play a role in the pathogenesis of SLE. Thus, the polyspecificity of monoclonal anti-DNA antibodies and synthetic polypeptide antigens suggests that antigens other than normal self antigens serve as immunogenic triggers.

SLE is a non organ specific autoimmune disease and as seen earlier, a large panoply of autoantibodies have been identified in the serum of lupus patients in varying frequency. The clinical picture of SLE shows malar and discoid rashes, photosensitivity, oral ulcers, non-erosive arthritis, pleuritis, pericarditis, hemolytic anaemia, lymphopenia and thrombocytopenia.

Anti-DNA antibodies play a major role in the development of lupus nephritis in MRL/lpr mice. Although anti - dsDNA antibodies are considered to be the hallmarks of human lupus nephritis, most of the anti-DNA antibodies in the murine lesions also bind to ssDNA (Gavalchin et al., 1987). Before the onset of disease, the production of anti-DNA antibodies shifts markedly from IgM to IgG isotype (Papoian et al., 1974). This switch is also associated with an increased cationic charge of the anti-DNA antibody production (Batta et al., 1987). The majority of anti - DNA antibodies in the renal lesions are of IgG2a and IgG2b subclasses (Gavalchin et al., 1987).

Purified forms of nucleic acids are poor immunogens (Stollar, 1973). Modification of DNA, for example by ultraviolet light exposure
(Davis et al., 1976) or the presentation to the immune system of DNA fragments (Lafer et al., 1981) has induced antibodies which bind to immunogen but not to unmodified B-DNA.

The anti-DNA antibody response of lupus mice resembles an antigen driven response that is composed of antibodies that are heterogeneous in their DNA binding specificity. More compelling evidence for the role of DNA as a selecting antigen has been obtained from the sequencing of panels of anti-DNA antibodies isolated from individual lupus mice (Schlomchik et al., 1990). Since the antibodies in these panels all bind to DNA and show clonal restriction, it may be concluded that DNA functions \textit{in vivo} as a selecting antigen. A microbe possessing both foreign epitopes and epitopes that mimic autoantigens has the potential to elicit an autoimmune response (Rekvig, 1995). Nucleic acids complexed with proteins have also been considered as potential immunogens. Many human monoclonal anti-DNA antibodies of both isotypes use VH genes which are members of fetally expressed repertoire. It is possible that these genes contain CDR sequences which lend themselves to formation of a DNA binding site. Comparison of sequences of large numbers of anti-DNA antibodies enables us to identify motifs which might contribute to such binding properties. Comparison of the sequence of antibody cDNA with that of the germline gene from which it is derived enables us to identify the positions and nature of somatic mutations which may likewise contribute to antigen binding (Isenberg et al., 1997).
Cardiovascular manifestations of SLE:

The cardiovascular manifestations of SLE have become more apparent with prolonged survival and improvement in diagnostic modalities. While most patients still die of infection or renal failure, the incidence of cardiovascular morbidity and mortality in SLE is rising, and is now the third leading cause of death in SLE (Urowitz et al., 1976). Whether there is a cardiomyopathy directly attributable to lupus is unresolved. Most series cite a high incidence of heart failure but attribute it to multiple problems, including; fever, infections, anaemia, uraemia, hypertension, steroids and accelerated atherosclerosis. Studies on systolic time interval in SLE patients indicate a shorter left ventricular ejection time and a longer pre-ejection period than control subjects, suggesting left ventricular systolic dysfunction, an indicator for primary lupus cardiomyopathy (delRio et al., 1978). Haemodynamic studies have revealed that lupus cardiomyopathy may exist even without clinical signs or may represent abnormalities of the intrinsic contractile and relaxation properties of the myocardium (Strauer et al., 1976). Various etiologies have been proposed for lupus cardiomyopathy. Das and Cassidy (1973) detected anti heart antibodies in 20 of 32 patients with SLE and proposed that these antibodies might be involved in the genesis of myocardial dysfunction in lupus. Bidani et al (1980) demonstrated immune complexes in the myocardium in 9 of 10 necropsies. They proposed that immune complex deposition led to complement activation, inflammation and myocardial damage. Either mechanism (anti heart
antibodies or immune complex deposition) may provoke the small vessel vasculitis, focal myocarditis, fibrosis and myocardial necrosis that is seen at autopsy in many SLE hearts. Further evidence is needed, however, to establish the link between these pathologic findings, immunologic abnormalities and cardiac dysfunction in vivo (Doherty and Siegel, 1985). Frustaci et al. (1996) have recently reported a case of lupus myocarditis causing left ventricular aneurysm. An immunologic mechanism of myocardial damage in this patient is suggested by the results of serologic studies (increased anti-nuclear antibodies, increased anti-DNA, decreased C3-4, increased anti-sarcolemmal antibodies) by the normal coronary arteriogram and by the cardiac biopsy which showed active myocarditis and fibrinous endocarditis. Post-mortem studies have revealed myocardial inflammatory lesions in up to 70% of victims of SLE. It remains to be seen if improved diagnosis and treatment of the cardiovascular manifestations of SLE can enhance survival.

**Objectives of the present study:**

Organ specific autoimmune disease are characterized by production of autoantibodies which react with autoantigens unique to that organ. Since in DCM, the only affected organ is the heart muscle, it is necessary that the criteria of an organ specific autoimmune disease is fulfilled. This includes genetic predisposition, presence of inflammation and abnormal HLA molecule expression in target organ, reproduction of human disease in susceptible animals with relevant
autoantigens and detection of organ and disease specific antibodies in affected patients and symptom free relatives. Although there is evidence that autoantibodies of DCM patients react to several different proteins, the epitopes for experimental autoimmune myocarditis and DCM still remain limited. Therefore, the cardiac muscle protein responsible for autoimmune myocarditis causing DCM must be identified.

In the present investigation, a systematic study was carried out to characterize the 150 kD C-protein autoantigen which is known to induce autoimmune myocarditis leading to DCM. The autoantigen, 150 kD human cardiac C-protein was characterized by ultraviolet spectroscopy, circular dichroism, thermal denaturation, SDS-PAGE and urea denaturation studies. The immunogenicity of C-protein was ascertained by inducing polyclonal antibodies in experimental animals. The repertoire of specificities of induced antibodies was assessed by direct binding and competition ELISA. Commercially available calf thymus native DNA was purified free of protein and single stranded regions and adducted to C-protein is presence of ROS and/or UV-B irradiation. The DNA C-protein adduct/photoadduct was characterized by nuclease S1 sensitivity assay. Kinetic behaviour of addition of lysine residues (present in C-protein) at various irradiation time was studied in case of DNA C-protein photoadduct. The recognition of C-protein and DNA C-protein adducts/photoadducts by DCM, SLE and induced anti C-protein antibodies was evaluated by direct binding and competition ELISA and band shift assay.