Review of Literature
REVIEW OF LITERATURE

Most living organisms have developed a remarkable system to protect themselves from invading pathogens and neoplastic cells. The immune system in the higher mammals can be broadly characterized as either “innate” or “adaptive”. The innate system does not require a prolonged period of exposure to pathogens and can act immediately, but does not generate long-lasting immunity. Phagocytes and Natural Killer cells are important components of innate immunity. When the innate immune system cannot get rid of the pathogen, adaptive immune responses come into play, with the generation of antigen-specific effector cells. These responses are characterized by the generation of immunologic memory and the capacity of self/non-self discrimination. Adaptive immune responses are mainly mediated by two cell types – the T and B lymphocytes. Defects in their functioning are often associated with serious pathological consequences; for example, anomalies in the ability to distinguish self from non-self could lead to autoimmunity, the pathological consequences of which result in autoimmune diseases. Autoimmunity per-se can occur without any overt disease or appearance of symptoms; it is only when the functions of targeted organs are adversely affected do autoimmune diseases occur. Thus, autoimmunity may be common in the general population; incidences of self-limiting, self-reacting T and B cells are high\(^1\), but only a small percentage of these individuals develop full-blown disease. Autoimmune diseases, described as *horror autotoxicus* by Paul Ehrlich, are therefore relatively rare.

Traditionally, autoimmune diseases have been classified as being either organ-specific (where a particular organ or gland is affected) or systemic (where multiple antigens and/or organs are targeted), although there exists an entire spectrum of pathologies between these two extremes. While Grave’s Disease is an oft-quoted example of an organ-specific autoimmune disease (where agonistic auto-antibodies target the TSH receptor), Systemic Lupus Erythematosus (SLE) is the prototypic systemic autoimmune disease, characterized by anti-self responses against a wide variety of antigens as well as the presence of multi-organ pathology; amongst the organs targeted are the kidneys, the brain, the lungs, and the heart.
Systemic Lupus Erythematosus

SLE (or lupus, as it is more commonly referred to) is a chronic inflammatory autoimmune disease of unknown etiology, affecting 1 in about 2,000 people\(^2\). Disease concordance in identical twins is 25-50\%, and in dizygotic twins around 5\%. There is also a higher risk of disease in the first-degree relatives of patients\(^3\) indicating the role of genetic factors in disease onset, as discussed is more detail below. Lupus predominantly affects women, especially of reproductive age. The female-to-male ratio is around 9:1 for patients between 15-50 years of age. Further, African-American and Hispanic-American women have a two-four times higher risk of developing the disease as compared to Caucasians\(^4\).

The disease progresses through four broad stages - the appearance of auto-antibodies against a number of ubiquitous self-antigens, the deposition of auto-antibodies and immune complexes in various tissues, tissue inflammation followed by tissue damage, and fibrosis affecting organ function, finally resulting in high morbidity and mortality\(^5\).

Lupus is the most heterogeneous autoimmune disease. Patients of SLE can manifest more than a hundred auto-antibody specificities. While some of these may be epi-phenomena, others are thought to cause (or are at least closely associated with) distinctive pathologies. Some individuals exhibit tissue deposition of auto-antibodies but no associated inflammation; others have antibody deposition as well as inflammation but no tissue damage and still others exhibit antibody deposition, tissue inflammation and associated pathology. Because of multi-organ involvement, a variable disease course and protean manifestations, diagnosis of SLE is often difficult. The American College of Rheumatology (ACR) has put forward eleven clinical criteria for diagnosis, which are detailed in Table 1\(^6,7\). A person found to exhibit four or more of these clinical conditions, either simultaneously or during any period of observation, is diagnosed as being afflicted. Employing these criteria, diagnosis can be achieved with 95\% specificity and 85\% sensitivity. In specific instances, the presence of three significant indicators (nephritis and the presence of anti-nuclear and anti-DNA antibodies) can also lead to a diagnosis of SLE.
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<thead>
<tr>
<th>CATEGORY</th>
<th>S.No</th>
<th>ITEM</th>
<th>DEFINITIONS</th>
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<tr>
<td><strong>Skin category</strong></td>
<td>1</td>
<td>Malar rash</td>
<td>Fixed erythema, flat or raised, over the malar eminences sparing the naso labial fold.</td>
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<td>2</td>
<td>Discoid rash</td>
<td>Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions.</td>
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<td>3</td>
<td>Photosensitivity</td>
<td>Skin rashes as a result of unusual reaction to sunlight, by patient history or physician observation.</td>
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<td>4</td>
<td>Oral ulceration</td>
<td>Oral or nasopharyngeal ulceration usually painless observed by physician.</td>
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<td><strong>Systemic criteria</strong></td>
<td>5</td>
<td>Non erosive arthritis</td>
<td>Involving two or more peripheral joints, characterized by tenderness, swelling or effusion.</td>
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<td>6</td>
<td>Pleuritis or pericarditis</td>
<td>Pleuritis: Convincing history of pleuritis or rub heard by a physician or evidence of pleural effusion</td>
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<td>OR</td>
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<td>Pericarditis: Documented by ECG or rub or evidence of pericardial effusion.</td>
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<td>7</td>
<td>Renal disorder</td>
<td>Persistent proteinuria greater than 0.5 g/day or greater than +3 if the equivalent not performed</td>
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<td>Cellular casts- may be red cells, hemoglobin, granular, tubular or mixed.</td>
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<td>8</td>
<td>Seizures or psychosis</td>
<td>Seizures- In the absence of offending drugs or known metabolic derangement. E.g. uremia, ketoacidosis or electrolyte imbalance</td>
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<td>OR</td>
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<td>Psychosis- In the absence of offending drugs or known metabolic derangement. E.g. uremia, ketoacidosis or electrolyte imbalance.</td>
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<td><strong>Laboratory criteria</strong></td>
<td>9</td>
<td>Hematological disorder</td>
<td>Hemolytic anemia with reticulocytosis OR Leucopenia- less than 4000/mm³ on two occasions OR Lymphopenia-less than 1500 on two occasions OR Thrombocytopenia- less than 100,000/mm³ in the absence of offending drug.</td>
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<td>10</td>
<td>Immunologic disorder</td>
<td>Anti-DNA: Antibodies to native DNA in abnormal titre OR Anti-Sm: Presence of antibodies to Sm nuclear antigen OR Positive finding of anti-phospholipid antibodies based on (a) An abnormal level of IgG or IgM anti-cardiolipin antibodies. (b) A positive test for lupus anticoagulant. (c) A false positive test for at least 6 months and confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test.</td>
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<td>11</td>
<td>Positive antinuclear antibody</td>
<td>An abnormal titre of antinuclear antibody by immunofluorescence or an equivalent assay at any point of time in the absence of drug.</td>
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Auto-antibodies

The most distinctive feature of SLE is the presence of auto-antibodies, mainly of the IgG isotype. Though more than a hundred different auto-antibody specificities have been described, those directed against nuclear antigens (such as dsDNA), phospholipids, ribonucleoproteins and RBCs have been the most extensively studied.

Anti-DNA Antibodies

Almost 99% patients of SLE (with or without nephritis) express anti-nuclear antibodies in their sera. Amongst them, approximately 50-70% patients carry anti-dsDNA antibodies. Though lupus nephritis can develop in absence of anti-DNA antibodies, in most of patients, the levels of anti-DNA antibodies increase during disease “flares”. These antibodies may form immune complexes with circulating DNA leading to complement activation, cross-linking of Fc receptors on leukocytes (as the pathogenic antibodies are mainly of IgG2a or IgG2b isotype) and inflammation. These complexes and/or antibodies are also thought to deposit in various organs like the skin, the lungs, on blood vessels and the kidneys. Deposition in the kidneys is thought to result in the onset of glomerulonephritis and the subsequent development of chronic renal failure. However, not all anti-DNA antibodies are pathogenic i.e. not all induce glomerulonephritis. In some instances, the pathogenic anti-DNA antibodies have been shown to react to glomerular antigens; α-actinin, a 100 KDa protein and a major component of glomerular podocytes, was recognized by such antibodies, while non-pathogenic anti-dsDNA antibodies showed either very weak binding or no binding to the protein. α-actinin mimics the charge characteristics of DNA, itself being very acidic, with many negatively charged amino acids. Sera and kidney eluates from the lupus-prone mice showed the prevalence of anti-α-actinin antibodies. Intravenous immunization in non-autoimmune animals with anti-DNA antibodies resulted in glomerular binding within one hour. The fact that these antibodies can cross-react with other auto-antigens such as Sm, may aid in their pathology.

The involvement of the central nervous system in lupus is reflected by the occurrence of cognitive impairment, depression, seizures and psychoses. It has now been shown that the pathogenic anti-DNA antibodies can cross-react with the N-methyl-D-aspartate NMDA subunit NR2 glutamate receptor in the central nervous system (CNS). Such antibodies could also bind neurons of the
cortex \textit{in vivo} and cause excitotoxic death in the neurons by the upregulation of caspase 3\textsuperscript{15}. \textit{In vivo}, intentional breakdown of blood-brain barrier permitted these auto-antibodies to gain access to the brain where they bound hippocampal neurons, causing death. Animals displayed performance deficits on tasks that depend on the integrity of the hippocampus. Antibody-mediated damage could be prevented by memantine, an NMDA receptor antagonist which is a potent non-competitive inhibitor of NR2A and NR2B-containing receptors\textsuperscript{16}.

Although T cells are not thought to play a direct role in tissue damage in SLE, CD4\textsuperscript{+} T cells seem to be required for the production of pathogenic IgG antibodies\textsuperscript{2}. Ample evidence exists describing the importance of somatic mutations in complementarity determining regions (CDR) for antibody specificity. High affinity anti-DNA antibodies (both pathogenic and non-pathogenic) have been shown to contain a relatively higher number of arginine residues in the variable regions of the heavy chain. Addition of arginines in the CDR3 has been shown to increase affinity to DNA; conversely, replacement of arginine by glycine (disrupting hydrogen bonding or electrostatic interactions) resulted in the partial or complete loss of binding\textsuperscript{17}.

Immunization with anti-dsDNA antibodies bearing the public idiotype 16/6 in non-autoimmune animals resulted in generation of autoimmune responses. This phenomenon has been discussed in greater detail below.

\textit{Anti-Ribonucleoprotein (RNP) antibodies}

Anti-RNP antibodies are a common occurrence in lupus patients. Such auto-antibody responses can be of the IgG, IgM\textsuperscript{18} and the IgA\textsuperscript{19} isotype and include antibodies to Ro/SSA, La/SSB and Smith (Sm) proteins. Anti-Ro antibodies are found in around 50\% of SLE patients and comprise antibodies directed against two non-homologous auto-antigens - Ro52 and Ro60; these can be found in other autoimmune diseases as well, such as Sjogren’s syndrome\textsuperscript{20}. Ro60 has a molecular mass of 60 KDa, is an evolutionary conserved molecule and is involved in the quality control of 5S rRNAs. It is present in both the cytoplasm and the nucleus, whereas Ro52 (52 KDa) is mainly found in the cytoplasm\textsuperscript{21}. The function of Ro52 is controversial, as is its association with Ro60\textsuperscript{20}. It has been shown recently in humans that antibodies to Ro60 can be detected as early as nine years before the symptoms of lupus appear\textsuperscript{22}, and earlier than the appearance of other auto-antibodies,
such as those against Sm. Children born to mothers harboring anti-Ro antibodies have a higher probability of developing congenital heart block (CHB)\textsuperscript{23}; around 2-5% females suffering from SLE deliver new-born babies with CHB. Conversely, nearly all infants with CHB are born to mothers harboring anti-Ro antibodies\textsuperscript{24}. Antibodies to Ro52 have been shown to bind cardiomyocytes and cause an intracellular overload of calcium concentrations, leading to loss of contractibility and eventual apoptosis\textsuperscript{25}. Reports have shown that Ro60 and La associate together in apoptotic blebs\textsuperscript{26}. During developmental processes, the cardiocytes that undergo apoptosis are quickly taken up by healthy cardiocytes. Maternal anti-Ro and anti-La antibodies inhibit uptake. It is postulated that inhibition of apoptotic cell uptake during massive regeneration can have serious pathological implications\textsuperscript{27}.

The La protein has a molecular mass of 48 KDa and plays a role in the termination of the transcription by RNA polymerase III and also allows for the multiple re-initiation of transcription by RNA polymerase III. It is mainly found in the nucleus and has been shown to be exported to the cytoplasm under conditions of stress\textsuperscript{28}. Auto-antibodies to La are present in around 34% SLE patients and appear later in the disease than anti-Ro antibodies. Like anti-Ro antibodies, anti-La antibodies are implicated in CHB. These antibodies bind La that is translocated to the apoptotic blebs of developing cardiomyocytes that die due to calcium overload (induced by anti-Ro antibodies), thus increasing the antibody deposition in the heart. Mortality due to CHB this is around 30% and more than two-thirds of the patients need life-long pacemaker implants\textsuperscript{29}.

The Sm ribonucleoproteins are a part of the spliceosomal complex. A number of Sm proteins are known: Sm B, B', D1, D2, D3, E, F and G. They function to remove the intronic mRNA. The presence of anti-Sm antibodies are specific to lupus and occur in around 30% of patients\textsuperscript{30}. These antibodies appear relatively late, just a few months before the diagnosis of the disease\textsuperscript{22}.

Twelve percent of SLE patients carry serum antibodies to ribosomal (Rib) P proteins; the incidence of this reactivity appears to be higher in patients also expressing anti-Sm antibodies. Anti-Rib P antibodies, like anti-Sm antibodies, are specific to lupus. Antibodies are mainly of IgG2a isotype, though IgG2b, IgG3 and IgG1 isotypes can also exist\textsuperscript{30}. Rib P proteins comprise of a family of acidic phosphoproteins P0, P1 and P2 with molecular masses of 38 KDa, 19 KDa and
17 KDa respectively, and auto-antibodies are directed mainly to the carboxy terminal of these proteins. Some reports suggest that the presence of these antibodies correlates with disease activity, and also with neuro-psychiatric manifestations (such as psychosis and depression) seen in lupus\(^{31,32}\). Anti-Rib P Protein antibodies can be poly-reactive, demonstrating binding to auto-antigens such as ssDNA, dsDNA and β2-glycoprotein-I (β2-GPI). In addition, an association between anti-Rib P Protein antibodies and anti-phospholipid antibodies has been recently demonstrated\(^{33}\).

**Anti-phospholipid antibodies**

Auto-antibodies against phospholipids (aPL) are also relatively frequent in lupus\(^{34}\) and are associated with thrombotic complications\(^{2,35}\). In the course of disease, anti-phospholipid antibodies appear at around the same time as anti-Ro and anti-La antibodies and are usually directed against negatively-charged phospholipids like cardiolipin and phosphatidylycerine. Lesser auto-antibody reactivity can also be seen against phosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol. Anti-phospholipid antibodies of the IgG, IgM or IgA isotypes have been described\(^{35,36}\). Some antibodies may be directed against PLs complexed with PL binding proteins such as β2-glycoprotein I (β2-GPI), also called apolipoprotein H\(^{37}\). Women with aPL suffer pre eclampsia, recurrent fetal loss, hemolysis, elevated liver enzymes and low platelet counts\(^{38}\). These antibodies are thought to bind to the surface of the platelets and endothelial tissue and in turn, cause decreased secretion of prostacyclin production by endothelium and increased thromboxane production by platelets, resulting in vasoconstriction\(^{37}\). Passive administration of monoclonal anti-cardiolipin antibodies in mice resulted in recurrent fetal losses and thrombocytopenia\(^{38}\). Auto-reactive β2-GPI specific CD4\(^+\) T cells have also been identified that do not recognize the native molecule but the reduced or recombinant β2-GPI produced in bacteria. It is hypothesized that cryptic epitopes (as discussed later) of β2-GPI initiate the auto-reactive response to phospholipids\(^{39}\). Hence, animals immunized with foreign β2-GPI develop anti-phospholipid antibodies. This phenotype could be transferred to naive mice by infusing the bone marrow from β2-GPI immunized animals. But when bone marrows were depleted of T cells, no auto-reactive anti-phospholipid responses were observed. This observation further strengthened the involvement of T cells in autoimmune responses to phospholipids. Accumulating reports now suggest that anti-phospholipid antibodies bind the placenta and the decidua, and activate
complement, resulting in the death of these tissues, finally resulting in fetal loss. When sub-optimal doses of heparin were used, binding of auto-antibodies was not affected but apoptosis of the cells could be inhibited by preventing complement activation both in vitro and in vivo\(^\text{40}\).

**Antibodies directed at cell surface proteins**

Auto-antibodies directed against cell surface molecules result in pulmonary hemorrhage, platelet destruction and endothelial damage\(^\text{11,37}\). The anti-phospholipid antibodies can cross-react with the red blood cell membrane and cause autoimmune hemolytic anemia (AIHA)\(^\text{41,42}\). In patients with high titres of anti-cardiolipin antibodies, hemoglobin (Hb) concentrations were very low, thereby exhibiting significant negative correlation\(^\text{41}\). In addition, antibodies directed against RBC surface antigens (Rh antigens such as E and c proteins\(^\text{43}\), and antigens of the Kell blood group\(^\text{44}\)) result in AIHA, also associated with the release of intracellular Hb. In the case of NZB mice that spontaneously develop autoimmune hemolytic disease, there exist two major groups of anti-mouse red blood cells (RBC) auto-antibodies. Antibodies of the first group are predominantly IgG and react with an exposed surface determinant (referred to as antigen X) on intact RBC; antibodies of the second group are of IgM class and react with the HB antigen only after treatment of RBCs with proteolytic enzymes\(^\text{45}\). Antibodies directed against the RBC surface cause hemolytic anemia due to Fc receptor-mediated erythrophagocytosis or sequestration of agglutinated RBCs in the spleen and the liver. These auto-antibodies, present on the cell surface of RBCs, can be detected by adding species-specific secondary antibody; agglutination is referred to as a positive “Coombs’ test”. The monoclonal anti-RBC antibody generated from Coombs’-positive old New Zealand mice (NZB) animals caused autoimmune hemolytic anemia in non-autoimmune animals BALB/c mice\(^\text{46}\). Another commonly targeted molecule is the Band 3 anion transporter protein in humans. Anti-RBC antibodies can be of IgG, IgM or IgA isotype. Though similar auto-antibodies can be found in healthy individuals, higher concentrations of these antibodies can be eluted from the RBC surface in autoimmune patients; thus, there appears to be a defect in the regulation of the natural anti-RBC antibodies in patients\(^\text{47}\).
Etiology and Pathology

Host genetics and environment are both thought to contribute towards the development of lupus. Though the primary cause of the disease remains unknown, a number of hypotheses have been put forward in efforts to help delineate its initiation and progression. These include the loss of central or peripheral tolerance, molecular mimicry with infectious agents such as bacteria and viruses, the exposure of cryptic epitopes, epitope spreading to include previously untargeted moieties and dysregulation of the idiotypic network.

Defects in tolerance mechanisms

Errors in tolerance have been shown to be responsible for various autoimmune manifestations. It has been demonstrated that, in healthy individuals, T cell populations that use the Vβ11 TCR family form a minor population in the thymus and in peripheral lymphoid tissues as a result of clonal deletion. In mice thymectomized one to four days after birth, their numbers in the periphery increase ten-fold, coinciding with the development of autoimmune diseases. Additionally, Aire (Autoimmune Regulator) has been shown to be important for central tolerance. It induces the expression of a number of peripheral-tissue antigens in thymic medullary epithelial cells which results in the negative selection of potentially auto-reactive T effector cells. In the absence of Aire, autoimmunity and ultimately overt autoimmune disease develops (Figure Ia).

Tolerance in the B-cell compartment arises through receptor editing, deletion and anergy. The strength of the B-cell receptor signaling and the nature of the antigen are important in determining the fate of B cells. A defect in any of the mechanisms involved in the maintenance of tolerance can lead to the generation of high number of auto-reactive antibodies in the bone marrow and in germinal centers (Figure Ib, Ic).

Molecular mimicry

In addition to other triggers, evidence implicating the role of infectious agents as initiators of lupus-specific autoimmune responses has emerged. The role of Epstein Barr virus as the initial insult to the immune system is being increasingly suspected. In a group of one hundred and seventeen SLE patients, it was found that one hundred and sixteen were sero-positive for EBV, whereas viral DNA was detectable in peripheral cells in all one hundred and seventeen patients.
When an Epstein Barr Nuclear Antigen-1 (EBNA) expression vector was injected into mice, the animals generated antibodies not only against EBNA-1 but also against dsDNA and the auto-antigen Sm; as indicated above, both reactivities are specific to lupus\textsuperscript{57}. It is believed that molecular mimicry between the EBNA-1 and the Sm nucleoprotein is responsible for these observations; a proline-rich epitope (PPPGMRPP) in the C-terminus of SmB'/B is similar to an epitope (PPPGRRP) in EBNA-1; the latter peptide is recognized by human anti-Sm sera\textsuperscript{58}. It is believed that EBV works by latently infecting B cells, promoting their proliferation and hyper-activation and encoding proteins that inhibit apoptosis (another hypothesized defect in SLE). In further evidence implicating EBV in lupus, antibodies directed towards a peptide representing amino acids 169-180 of the auto-antigen Ro60 cross-react with a peptide representing amino acids 58-72 of EBNA, even though the two peptides exhibit no sequence homology\textsuperscript{59}.

**Crypticity**

In some instances, tissue-specific antigens are sequestered and are thus unavailable to the immune system. In addition, while T cells against dominant self-determinants are eliminated or rendered tolerant, those against other epitopes may escape tolerance. Determinants which are quiescent when present as part the whole molecule, but immunogenic when presented by themselves, are referred to as “cryptic”. It is postulated that viral infection (along with attendant inflammation and the enhancement of co-stimulatory functions) may result in the effective presentation of previously sequestered or cryptic antigens, leading to the activation of previously quiescent T and B cells\textsuperscript{48,49}. Indeed, immunization with a cryptic peptide can lead to autoimmunity\textsuperscript{60}, a phenomenon which usually requires inflammatory conditions\textsuperscript{1}.

**Epitope spreading**

As indicated above, it has been shown in human SLE patients that several years before the onset of the disease, there occur auto-reactive antibody responses directed against amino acids 169-180 of Ro60. As time progresses, other regions of the molecule are targeted, a phenomenon known as intra-molecular epitope spreading\textsuperscript{59}. Additionally, immunization of rabbits with the EBNA-1 peptide cross-reactive this Ro60 peptide (described above) also leads to epitope spreading to the other Ro60 peptides. Immunization with a dominant T cell epitope of La led to the appearance of antibodies to both Ro60 and La\textsuperscript{61}. Immunization with either Ro60 or La led to the appearance of
autoimmune responses to both La and Ro60\textsuperscript{62}. In addition, immunization of mice with a dominant epitope of the Sm antigen lead to the appearance of antibodies directed to other parts of the molecule\textsuperscript{63}. The phenomenon is explained thus: Were B cells of different specificities to internalize multi-component protein complexes (after recognition of their respective cognate epitopes) and then receive help from a single T cell against which tolerance has been broken, antibodies of different specificities would be induced (Figure Ih). However, emerging data indicate that this model may be overly simplistic.

**Idiotypy**

In 1974, Jerne proposed that the immune response might be regulated by antigenic determinants on the immunoglobulin variable region (idiotypes)\textsuperscript{64}. Data has revealed that the immune system may be involved in the progression of autoimmune disease. Immunization with anti-DNA antibodies (Ab1) bearing the “pathogenic idiotypic” 16/6 generated Ab2, which in turn endogenously generated Ab3; Ab3 bore the 16/6 idiotypic and demonstrated anti-DNA specificity, thus perpetuating disease. Unexpectedly, immunization also led to high titers of anti-ssDNA, anti-dsDNA, anti-Sm, anti-RNP, anti-Ro and anti-La auto-antibodies\textsuperscript{65}. Immunization with antiacardiolipin antibodies was shown to lead to anti-phospholipid syndrome (APLS)\textsuperscript{66}. Immunization of rats with a poly-reactive human monoclonal antibody directed against apoptotic cells led to the generation of anti-idiotypic antibodies targeting molecules distinct from those recognized by the immunizing antibody\textsuperscript{67}.

**Generation of Neo-Epitopes**

Another theory suggests that autoimmunity can arise upon the formation of neo-self determinants, generated through the binding of drugs or other haptenic groups to self molecules, as well as through molecular modifications via gene mutations\textsuperscript{49}. A study on monocytes from SLE patients indicated impairment in the removal of 8-oxodG formed due to oxidation by reactive oxygen species (ROS) in the DNA\textsuperscript{68}. It has been reported that anti-DNA antibodies found in SLE demonstrate enhanced recognition of ROS-modified DNA\textsuperscript{69}. 
Figure 1: (a) Although normal T cells exposed to self-antigen in the periphery become tolerized, lupus-prone T cells are sensitive to lower thresholds of activation by agonist or weak-agonist peptides. (b) Once activated, T cells provide primary stimulation to genetically hyper-responsive B cells. (c) These auto-antigen-stimulated B cells undergo somatic hypermutation and affinity maturation. (d) On the synthesis of pathogenic auto-antibodies, tissue damage results in the release of self-antigen, (e), (f) which is taken up and presented by specific antigen-presenting B cells in a second round of T-cell activation, (g) leading to a positive-feedback cycle. (h) Autoimmune T-and B-cell responses are diversified, which results in epitope spreading. This continuing and cyclic process of B cell-T cell cognate interaction serves to amplify the ensuing autoimmune processes. (i) Activated T cells can also directly cause tissue pathology by migrating to the target organ and releasing cytokines and by mediating direct cytotoxicity. T cells are shown orange; B cells are red.

Defects in apoptosis and apoptotic cell uptake

Under normal circumstances, apoptotic cells are efficiently engulfed by macrophages in the early stages of the apoptotic cascade, the process being anti-inflammatory in nature. Several lines of evidence indicate that defects in apoptosis and/or clearance mechanisms are pre-disposing factors for lupus. Reduced clearance of apoptotic bodies is observed in lupus patients, an effect attributed to intrinsic phagocytic defects. In addition, lupus patients demonstrate a higher percentage of apoptotic lymphocytes in circulation and higher rates of apoptosis are observed in short-term *in vitro* lymphocyte cultures, leading to increased release of nucleosomal material.

Clq deficient mice develop lupus-like symptoms and demonstrate a higher preponderance of apoptotic bodies in the glomeruli, indicating either increased apoptosis and/or inefficient clearance of apoptotic bodies. Consequent to excess apoptosis and impaired clearance, post-apoptotic necrosis occurs, leading to modification of molecules present in the apoptotic bodies and the creation of neo-epitopes. Proteolytic cleavage of these neo-antigens may lead to the exposure of cryptic epitopes, breakdown of tolerance, causing the spread of immune responses to other auto-antigens.

In animals treated with clodronate liposomes to induce the apoptosis of macrophages, the kinetics of the appearance of proteinuria and severe glomerulonephritis were enhanced. These animals also had higher levels of total IgG levels. Somewhat paradoxically, defects in the apoptotic cascade due to the loss-of-function mutations in the *fas* gene or the *gld* gene (which encodes for Fas-ligand) also results in a lupus-like syndrome in mice and humans.

Role of hormones

It has been established that host genetics and the environment both contribute towards pathological sequelae. As mentioned earlier, the incidence of disease is the highest in women in the reproductive age group, leading to the hypothesis that lupus onset and progression may, at least in part, be influenced by the hormonal milieu.

SLE male patients have significantly higher levels of the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). In addition, some subjects also demonstrate lower
testosterone levels\textsuperscript{77}. Female lupus patients exhibit decreased levels of other androgens (androstenedione, dehydroepiandrosterone and dehydroepiandrosterone sulfate). Significantly, the lowest levels of these hormones were found in female patients with active disease\textsuperscript{78}. Abnormal estrogen metabolism (conversion of estrogen to 16α hydroxylated estrogen) has also been reported; 16α hydroxylated estrogen metabolites are the more potent and feminizing estrogens\textsuperscript{79}. An increase in the aromatase activity has also been observed, which would contribute to the imbalance between androgen and estrogen levels, since aromatase is involved in the synthesis of estrogen; higher aromatase levels are directly correlated to plasma levels of estrogen\textsuperscript{80}. Thus, excess estrogenic activity and low androgenic activity might contribute to disease pathology, a hypothesis strengthened by the fact that disease "flares" occur during periods of high circulating estrogen, for example during pregnancy and after exogenous administration of the steroid\textsuperscript{81}.

Prolactin levels were also found to be higher in the non-stimulated peripheral blood mononuclear cells (PBMCs) from lupus patients when compared with the healthy controls\textsuperscript{82}.

\textit{Environmental factors}

Amongst the environmental factors that might be responsible for the initiation of the disease are the infectious agents (as discussed earlier), dietary supplements such as alfalfa sprouts which contain L-canavanine\textsuperscript{37}, drugs such as procainamide and hydralazine and isoniazid in individuals that are slow acetylators\textsuperscript{83,84} and exposure to ultraviolet (UV) rays. It was observed that exposure of keratinocytes to UV-B (but not UV-A) led to the binding of antibodies present in the sera lupus patient sera to the surface of cells; monospecific anti-Ro and anti-La antibodies also bound to such cells, implying exposure of lupus-related auto-antigens\textsuperscript{85}.

A single environmental factor is not thought sufficient to cause disease. Evidence indicates that a combination of factors, coupled with a susceptible host genotype, may be responsible. A number of murine models of lupus exist. Extensive work using mice which express a lupus-like phenotype has helped further delineation of disease pathology and genetics.
**Mouse Models**

Several animal models of SLE exist, and have proved invaluable in investigations of disease processes. Autoimmune-prone mice are particularly relevant, since 99% of the mouse genome is homologous to the human genome and most of the immunological abnormalities apparently fundamental to the human disease are also operative in the mouse. A mouse chromosomal region containing a disease-susceptibility locus will generally have a syntenic region in the human genome. Three basic types of animal models have been used in autoimmune research:

1. Spontaneous models
2. Induced models
3. Genetically engineered models

The third category has been further divided into transgenic and knockout mice.

**Spontaneous Models**

These models are produced fortuitously; some were first described more than thirty years ago. For example, the chance crossing between New Zealand Black (NZB) female and New Zealand White (NZW) male mice produced one of the best-studied murine models for lupus-(NZB x NZW) F1 and the related New Zealand Mixed (NZM) 2410 congenic recombinant strain. Neither the NZB nor the NZW parents develop severe lupus-like symptoms. Other well-characterized models include MRL and BXSB mice. In (NZB x NZW) F1, the onset of autoimmunity involves multiple genes. In MRL and BXSB mice, immune dysfunction appears to be mediated by single-gene defects, though background genes may contribute to the ultimate phenotype; the former carry a mutation in the Fas gene, and the latter contain the Y-linked Yaa gene (discussed in detail later). These genes were identified by chance; they were inherited across generations in a simple Mendelian fashion and affected apoptosis in mice with lymphoproliferation (for MRL) or were linked with sexual dichotomy (for Yaa). Although the specific features of disease vary among these models, all are characterized by the development of high titres of IgG auto-antibodies to nuclear antigens, including dsDNA. Mice suffer from glomerulonephritis and extra-renal manifestations such as thymic atrophy, splenomegaly, hemolytic anemia, vasculitis and arthritis.
(a) \((NZB \times W) F_1\): This was the first lupus model to be developed. NZB and NZW were derived independently in New Zealand in the 1950s; NZB animals were initially noted to develop hemolytic anemia\(^{92}\). NZW were first described as being disease-free; glomerulonephritis was documented in females in subsequent studies, in the absence of increased mortality\(^{90}\). In 1963, \(F_1\) hybrids between these two strains were reported, which developed renal pathology very similar to that seen in human lupus. Disease shows a strong female bias, and the kinetics of the development of auto-antibodies mimics that seen in human lupus; end-organ failure due to tissue deposition of antigen-antibody complex is frequently seen. Recent studies have shown that more than twenty loci are involved in the development of disease\(^{93}\). Glomerulonephritis first occurs at four-five months, along with proteinuria. \(F_1\) mice are heterozygous at \(H2\) locus \(H2^{ds}\), inherited from NZB \(H2^d\) female and NZW \(H2^s\) male and it is believed heterozygosity is associated with auto-antibody production\(^{94}\) and increased frequency of glomerulonephritis\(^{95}\). Genes from both the parents contribute to the development of disease\(^{94,96}\). Various disease susceptibility loci have been identified; \(sle1, sle2, sle3, sle5\) and \(sle6\). Four suppressor loci have also been mapped - \(sles1, sles2\) and \(sles3\) and \(sles4\). These loci appear to interact with each other as well as with other loci in an epistatic fashion to contribute towards final disease outcome\(^4,97,98\).

The NZM strain was derived in 1980 by the breeding between NZB/W \(F_1\) hybrid female with an NZW male. Due to recombination of the ancestral gene, a litter of mixed coat colour was developed. Some of the descendents succumbed to early renal failure. A number of lines were generated, such as NZM 2410, NZM 64 and NZM 2758, with NZM 2410 being closest in terms of disease symptoms to human lupus - animals develop nephritis, proteinuria, and immune complex deposition in the brain, along with other associated pathologies\(^99\).

(b) \(MRL^{lpr/lpr}\): \(lpr\) was identified as a spontaneous mutation of the Fas (CD95) molecule and \(gld\) (Generalized Lymphoproliferation Disease) a mutation in the Fas ligand (FasL) in mice. Fas and FasL belong to tumor necrosis factor (TNF) and TNF-receptor (TNF-R) receptor family respectively. Active FasL exists as a homotrimerized complex which, on binding membrane-bound Fas, causing the latter's oligomerization; apoptotic death of Fas-bearing cells follows\(^{100}\). Homozygosity of either the \(lpr\) and \(gld\) mutations results in lupus-like disease, as well as in the accumulation of \(CD4^+CD8^-\) CD3\(^+\) T cells\(^{101}\). The autoimmune phenotype is most apparent when
the Fas mutation is on an MRL background, which is an admixture of LG/J (75%), AKR/J (12.6%), C3H (12.1%) and C57BL/6 (0.3%)[^89]. In 1967, a similar mutation was described in a human patient, leading to disease now known as autoimmune lymphoproliferative syndrome (ALPS)[^100]. Self-reactive T and B cells, arising due to defects in the apoptosis pathway caused by these mutations, are thought to contribute to disease[^102]. Though “background” genes are clearly important (for example, MHC haplotypes which help in the shaping autoimmune T cell repertoire), such genes by themselves are insufficient to cause lupus, as they can be shared by non-autoimmune individuals[^103].

The Fas mutation has been mapped to chromosome nineteen, and arises due to the insertion of a transposable element between exons two and three, causing aberrant RNA splicing and premature termination of Fas gene transcription[^104]. Aberrant FasL expression, on the other hand, is associated with normal transcription of the gld gene, but the lack of a functional FasL product due to a single amino acid change at the C-terminal portion of the molecule[^103]. Defects in functional lpr and gld protein expression should be considered accelerators; though mutations on an otherwise normal (non-autoimmune) background do induce the generation of auto-antibodies, minimal histopathology is observed. In addition, aging MRL^+/+^ mice demonstrate the presence of auto-antibodies as well. Other genes that might be implicated in the pathogenesis associated with lupus in MRL/lpr are now being slowly unraveled; experiments with chemokine receptor Ccr2 deficient MRL/lpr mice show that these animals developed less adenopathy and demonstrate a lower percentage of CD4+ and CD8+ peripheral T cells[^105]. Recent reports have implicated various loci such as Lprm4 on chromosome five, Ldrl1 on chromosome seven and Asm on chromosome ten[^106].

(c) BXSB: These mice are derived from a cross between a C57BL/6J (H-2^b^) female and a SB/Le (H-2^b^) male[^88]. They have a mutation in the Y-linked autoimmune acceleration (Yaa) gene that is responsible for the accelerated onset of lupus-like disease[^107]. Since males develop disease earlier than females, it was hypothesized that the autoimmune-enhancing phenotype was being encoded by the Y chromosome derived from the SB/Le strain[^108]. It has been shown that the genetic lesion underlying Yaa is an X to Y translocation of a telomeric region of more than 1 Mb, resulting in the duplication of around sixteen genes, including the gene for Tlr7[^109], which has been implicated in the development of lupus. Addition of Yaa gene to other autoimmune backgrounds by cross-
breeding results in worsening of disease. The detailed molecular mechanisms related to disease onset are still under study.

**Induced Models**

These models helped further elucidate various aspects of lupus pathogenesis.

(a) **Peptide-induced models:** Injection of dsDNA (a major auto-antigen) does not result in auto-antibody production\textsuperscript{110}. In an effort to identify a peptide surrogate of dsDNA, panning of a random phage display library using a murine anti-dsDNA antibody identified the peptide DWEYSVWLSN. Immunization of non-autoimmune BALB/c mice with this peptide resulted in the generation of high titres of IgG antibodies (mainly IgG1) against dsDNA. Auto-antibodies were also generated against histones and cardiolipin. In addition, increased immunoglobulin deposition was observed in renal glomeruli\textsuperscript{111}. When peptides aa26-40 and aa56-70 of the auto-antigen snRNP D protein were used to immunize normal mice, strong autoimmune T cell and B cell responses were observed against other snRNPs\textsuperscript{112}. The auto-antibodies also demonstrated anti-nuclear reactivity. Interestingly, no autoimmune responses were observed when the whole protein was used for immunization, indicating crypticity of the peptides. Similar responses were also seen with Ro60 peptides. When Ro60\textsubscript{316-335} peptide was used for immunization, immune responses were seen against other regions of Ro60 and also against other ribonucleoproteins such as La, SmD, 70-KDa U1RNP and the Golgi apparatus, which could not be absorbed by the immunizing peptide\textsuperscript{113}. Peptide PPPGMRPP (which is repeated four times in Sm B/B', and is a major auto-antigenic epitope in human patients) when immunized in a number of mice strains, resulted in the development of high titers of anti-Sm antibodies, with epitope spreading observed to other regions of Sm B/B' and Sm D, and the appearance of anti-nuclear antibodies as well\textsuperscript{114}.

(b) **Pristane-induced autoimmunity:** Pristane (2, 6, 10, 14-tetramethylpentadecane), when injected intraperitoneally in non-autoimmune BALB/c mice, induced auto-antibodies characteristic of lupus, such as IgM anti-ssDNA antibodies and anti-histone antibodies. However, no anti-dsDNA antibodies were observed. Injected animals also demonstrated anti-Sm responses, in conjunction with significant proteinuria, supporting the idea that the lupus phenotype arises as a result of interplay between genetic and environmental factors\textsuperscript{115}. In SJL mice injected with pristane, auto-
antibodies were generated against ribosomal protein P and animals went on to exhibit extensive glomerulonephritis\textsuperscript{116}.

(c) \textit{Idiotyp}y: A lupus-like syndrome resulted when a human monoclonal anti-dsDNA antibody (bearing the "public" 16/6 idiotype, discussed previously) was immunized in non-autoimmune C3H.SW female mice; high levels of anti-16/6 and anti-anti-16/6 antibodies were observed, with concomitant increase in anti-DNA antibodies. Elevated titres of auto-antibodies reacting with the ssDNA and ribonucleoprotein auto-antigens Sm, Ro and La, as well as with cardiolipin were observed. Increased leucopenia and proteinuria were also noted. Additionally, immune complexes of IgG and IgM that were deposited in the kidneys contained the 16/6 idiotype\textsuperscript{110}. Immunizations with 4B4 (an anti-Sm human monoclonal antibody) led to the production of antibodies against dsDNA, ssDNA, Sm and the mouse Fc fragment, though no antibodies could be detected against Ro and La. No inflammatory changes were observed in kidneys\textsuperscript{117}. In another study, immunization with peptides derived from the variable region of the heavy chain of the anti-DNA antibody Id540 and A6.1 were found to increase the survival, delay the development of nephritis and anti-double stranded antibodies\textsuperscript{118}. Immunization of an apoptotic cell-specific antibody resulted in the generation of anti-idiotypic antibodies exhibiting reactivity to antigens different from that of the immunizing auto-antibody, further indicating that the idiotypic network could be potentially be involved in antigen spreading and disease progression\textsuperscript{67}.

\textit{Genetically engineered models}

These models are used for the investigation of molecules of potential relevance to lupus progression and pathogenesis; genes of interest are either transgenically expressed or knocked out. Some of these are:

(a) \textbf{B-Cell Lymphoma Protein-2 (Bcl-2):} Bcl-2 is 24 KDa protein which promotes cell survival by the suppression of apoptosis. Bcl-2 transgenic mice, where expression is restricted to B lymphocytes, exhibit excess B cells, pre-B cells and plasma cells (attributed to their increased longevity) associated with heightened serum immunoglobulins. Plasma cells were detected in cultures even after 3 weeks; cells from non-transgenic strains died within 6 days. Animals had
amplified and protracted antibody responses and by one year of age, 60% of the animals exhibited anti-nuclear antibodies, glomerulonephritis, lymphadenopathy and myocardial infarction.

(b) FcγRIIB: This molecule acts as a negative regulator of immune-complex triggered activation, and functions \textit{in vivo} to suppress autoimmunity by regulating B-cell function. Loss of this inhibitory receptor is a B-cell autonomous defect. These animals showed increase in the number of IgG+ plasma cells leading to development of pathogenic IgG anti-DNA antibodies, autoimmune glomerulonephritis with C3 and antibody deposition in kidneys in a strain-specific manner. Deletion of the gene in the C57BL/6 background resulted in elevated immunoglobulin responses to DNA, increased inflammatory response to immune complexes, glomerulonephritis and decreased survival. In human lupus patients, the memory B cells have significantly lower surface expression of FcγRIIB.

(c) IFN-γR: Reports indicate that the production of anti-nuclear antibodies, glomerulonephritis and mortality is decreased by administration of neutralizing IFNγ antibodies or soluble IFNγ receptor in NZB/NZW F1 mice. IFNγR knockout animals also had lowered levels of pathogenic IgG2a and IgG3, in addition to suppressed IgM and IgG1, less Ig and C3 deposition in kidneys, and an absence of renal failure. In a contrasting report, another group demonstrated that IFN-γR deficient MRL^{lpr/lpr} mice, which expressed colony stimulating factor-1 (CSF) or granulocyte-macrophage colony stimulating factor (GM-CSF) in tubular epithelial cells, had four to five fold higher macrophage accumulation in the kidneys due to more rapid proliferation and decreased apoptosis, when compared with IFN-γR intact mice. There was also increased migration of dual negative (CD4+, CD8+) T cells.

(d) TNF-R1: Fas and TNF receptor-1 (TNF-R1) belong to the same family of genes. Tnfr1 receptor knockout mice do not develop autoimmunity. However, when these mice were crossed with mice of the MRL^{lpr/lpr} strain, F1 animals exhibited a five-ten fold increase in lymph node weight. The presence of auto-antibodies was observed; early-onset autoimmune disease accompanied by lesions in the kidney, lungs, liver and knee joints was associated with high mortality.
(e) TGF-β: Mice deficient in TGF-β exhibit massive lymphocytic and monocytic infiltration in the lungs, salivary glands, liver and the heart. Serum antibodies against dsDNA, ssDNA, Sm ribonuclear protein, collagen type I and collagen type V were present and Ig glomerular deposits were observed. There were no antibodies to the Ro and La ribonucleoproteins. Auto-antibodies to dsDNA and Sm were predominantly of the IgG isotype. These results support the concept that absence of suppressor cytokines can lead to systemic autoimmune disease. 

(f) C1q: Homozygous deficiency of the first component of complement pathway in humans is very strongly associated with the development of SLE. As discussed earlier, C1q has been shown to bind to apoptotic cells and promote their clearance, and homozygous deficiency in mice leads to the generation of anti-nuclear and anti-histone antibodies, immune complex deposition in kidneys, the presence of multiple apoptotic bodies in diseased glomeruli, and increased mortality. Crossing of C1q<sup>−/−</sup> and MRL<sup>+/+</sup> animals (which have an intact Fas gene) resulted in increased disease in F1 animal in comparison with MRL<sup>+/+</sup> animals; higher titers anti-nuclear antibodies (ANA) and anti-ssDNA antibodies were observed, along with kidney failure and proteinuria. Prevalence of glomerulonephritis was higher in females than in males. It has recently been shown that C1q deficiency increases the positive selection of auto-reactive B1b cells leading to the generation of high titres of IgM auto-antibodies directed against intracellular antigens.

(g) C4: C4 deficiency in mice lead to impaired antibody production. However, by five-six months of age, more than half of the animals demonstrated significant titres of ANA, with higher reactivity observed towards ssDNA than dsDNA. Glomerular pathology was observed at a higher incidence in females, with the demonstrated presence of IgG3 and C3 in the mesangia. Animals demonstrate elevated levels of circulating IgM-dsDNA complexes. Deposition of apoptotic dsDNA in splenic marginal zones is believed to induce secretion of IFN-α by CD11b<sup>+</sup> cells. C4 deficiency in conjunction with Fas deficiency results in significantly higher cervical lymph node mass on the C57BL/6 background; such animals also exhibited heightened glomerulonephritis, with increased deposition of IgG in the kidneys and ANA titer.
(h) **c-Mer**: The membrane tyrosine kinase domain of c-Mer helps in uptake of apoptotic cells. The animals lacking the kinase domain demonstrate low in-vivo clearance of exogenously administered apoptotic cells. There is a progressively increased level of auto-antibodies to chromatin and animals spontaneously develop auto-antibodies to IgG, chromatin ssDNA, dsDNA and display features of moderate renal pathology. Autoimmunity is not accompanied by polyclonal B cell activation in these animals.

(i) **Lyn**: Lyn is a protein kinase of the Src family expressed in B cells, monocytes and macrophages. It is involved in B cell receptor (BCR) mediated signaling. Lyn−/− deficient mice have reduced numbers of circulating lymphocytes and smaller or undetectable Peyer’s patches but paradoxically also suffered from splenomegaly and enlarged lymph nodes, in addition to having high concentrations of IgM and IgA in sera. Conversely, the numbers of Mac1+ positive cells (macrophages, neutrophils, NK cells and activated CD8+ cells) were increased in knockout animals, both in the spleen and lymph nodes. High titres of anti-dsDNA antibodies were observed, most of which were mediated by IgM. IgG immune complex deposition in kidneys was associated with glomerulonephritis.

(j) **BAFF**: B cell activation factor belonging to TNF family (BAFF) is a 285 amino acid transmembrane protein of TNF ligand super-family. It is cleaved on the cell surface to form a biologically active 17 KDa molecule and is involved in suppression of B cell apoptosis. Increased levels of circulating BAFF is observed in SLE patients. Over-expression of BAFF in mice was shown to lead to defects in B cell tolerance and SLE-like autoimmunity associated with increased antibody production to dsDNA, ssDNA and rheumatoid factor. The total serum levels of IgM, IgG2c, IgG2b, and IgG3 were significantly increased, leading to antibody deposition in the kidneys. In baff−/− mice generated upon the NZM 2328 background, the number of splenic B cells and CD4+ T cells were reduced by more than 90% and 70% respectively. The number of circulating mature B cells was reduced (though immature B cells numbers remained unaltered), resulting in a reduction in the level of circulating IgG. At six to seven months, kidney deposition of IgG was greatly reduced and glomerular C3 was barely discernible.
(k) **Dnase1**: Dnase1 is a major nuclease, involved in the degradation of DNA at sites of high cell turnover. Dnase1 deficient mice develop symptoms of severe lupus (including high ANA titres and glomerulonephritis) and die at six to eight months. Animals expressed high levels of antibodies against nucleosomes, ssDNA and dsDNA, in addition to antibodies to ribosomal protein P and histones. IgG and complement deposition was also evident in the kidneys\textsuperscript{142}.

(l) **PD-1**: PD-1 is a transmembrane protein belonging to the immunoglobulin super-family and contains the immunoreceptor tyrosine-based inhibitory motif (ITIM). It has been found to be strongly induced in lymphocytes following activation. Animals lacking the PD-1 gene develop mild proliferative glomerulonephritis at six months, significant deposition of IgG3 and IgM the glomeruli. By fourteen months, significant glomerulonephritis and arthritis is observed. PD-1 deficient animals additionally harboring the lpr/lpr genotype exhibit accelerated onset of disease\textsuperscript{143}.

(m) **Serum Amyloid Protein (SAP)**: SAP is a highly conserved plasma protein that shows specific, calcium-dependent binding to DNA and chromatin; it displaces H1-type histones, thereby solubilizing native chromatin. Binding reduces inter-nucleosomal cleavage; in the absence of SAP, DNA is rapidly cleaved. SAP deficient mice spontaneously produced high titres of antibodies against chromatin, DNA and histones. Female mice suffer from a high incidence of severe proliferative glomerulonephritis, immune deposition and mortality\textsuperscript{144}.

(n) **Secretory IgM**: Mice in which B cells lack the ability to secrete IgM develop lupus-like disease. These mice at four to six months demonstrate higher lymphoid organ mass and total cell numbers. This was accompanied by nephritis with significant cellular infiltrates comprising mainly of T cells\textsuperscript{145}.

**Cytokines**

From the human and animal studies mentioned above, as well as from other reports, it is clear that cytokines play key roles in the pathogenesis of disease\textsuperscript{146}. Altered levels of IL-1, IL-4, IL-6\textsuperscript{146}, IL-2\textsuperscript{147}, IL-10\textsuperscript{148}, IL-12\textsuperscript{149}, IFN-α\textsuperscript{150}, TNF-α\textsuperscript{151,152}, IFN-γ\textsuperscript{153} have been associated with lupus; alterations in type I interferons seem particularly significant\textsuperscript{148}.
Overproduction of IL-1 has been linked to pathogenicity in SLE. In II-1β−/− BALB/c animals, when lupus was induced by immunization with 16/6 idiotype, lower levels of anti-dsDNA antibodies and diminished disease was observed. Knockout animals showed lower levels of IL-2, IL-4, IL-1, IFN-γ and TNF-α secretion. IL-1β inhibition has been shown to suppress the spontaneous production of IgG from PBMCs from SLE patients. When PBMCs from healthy individuals are cultured with sera obtained from SLE patients, an upregulation of IL-1β mRNA is observed.

Apparently paradoxically, neutralization of IL-2 by anti-IL-2 antibody results in development of autoimmunity characterized by autoimmune hemolytic anemia, gastritis, splenomegaly, lymphadenopathy and multi-organ lymphocyte infiltration. Treatment reduces the numbers of FoxP3 expressing regulatory T cells; the resulting disease can be reversed by adoptive transfer of CD25+ CD4+ FoxP3 cells. Decreased in vitro secretion of IL-2 by CD4+ cells from SLE patients is often seen and correlates with high IgG levels in the serum. The hyposcretion of IL-2 can be reversed by resting T cells from the SLE patients for two-three days in culture before stimulation with mitogen. This correlates with the higher concentration of cAMP response element modulator (CREM) in the nuclei of the T lymphocytes from the SLE patients. CREM, which normally binds to the -180 site of IL-2 promoter, leads to decreased promoter activity and thus transcriptional repression of IL-2. When the normal T cells were treated with the sera from SLE patients, there was increase in the concentration of CREM mRNA and protein level. In another study, blocking of IL-2 mediated signal in the mice lacking FoxP3 (and thus the regulatory T cells) partially inhibited the lymphoproliferation and autoimmunity associated with the lack of regulatory T cells and resulted in prolonged life span versus the FoxP3 null animals.

IL-4, when constitutively expressed as a transgene in B cells, completely prevented the nephritis and mortality associated with lupus, probably by lowering of the levels of pathogenic IgG2a and IgG3 isotypes and increasing IgG1; levels of the glycoprotein (gp)70-anti-gp70 complex were also reduced. However, contradictory reports suggests that IL-4 knockout animals, while expressing reduced titers of IgG1 (with no changes in IgG2a and IgG2b) demonstrated reduced renal and salivary gland disease and decreased lymphadenopathy.
Lupus mouse models as well as SLE patients have high serum concentration of IL-6\textsuperscript{162,163}. Reports suggest that the IL-6 receptor is constitutively present on B cells from SLE patients, in contrast to B cells from healthy controls\textsuperscript{164}. Elevated levels of IL-6 were found in the cerebrospinal fluid (CSF) from patients with neuropsychiatric lupus erythematosus (NPSLE)\textsuperscript{165,166}. Oligodeoxynucleotide CpG, derived from sequences found in circulating DNA from SLE patients, caused enhancement of IL-6 and IL-8 mRNA from the endothelial cells \textit{in vitro}\textsuperscript{167}. Treatment of MRL\textsuperscript{1pr/1pr} animals with anti-IL-6 antibodies markedly decreased glomerulonephritis and caused a temporary reduction in anti-dsDNA antibodies\textsuperscript{168}.

IL-8 is a chemokine that appears to be a useful marker for central nervous system involvement in lupus; levels decrease during remission\textsuperscript{166}. Some reports suggest that the presence of IL-8 can be used for diagnosis of NPSLE\textsuperscript{165}.

Serum levels of IL-10 too have been found to be elevated in SLE patients, probably due to increased secretion by monocytes\textsuperscript{168}. The levels of IL-10 significantly correlate with disease severity; higher levels of the cytokine are seen in murine lupus as well\textsuperscript{169}. High IL-10 results in increased apoptosis in CD4\textsuperscript{+} cells, an effect blocked by anti-IL-10 antibodies\textsuperscript{170}. Monocytes from the SLE patients had decreased IL-12 production\textsuperscript{171}; levels of IL-12 go up during remission\textsuperscript{168}.

Plasma levels of IL-17 and IL-23 were higher in patients suffering from lupus than the healthy controls and there was a positive correlation with the disease severity. The \textit{ex-vivo} induction of PBMCs from SLE patients by IL-23 could significantly induce and augment the release of IL-17\textsuperscript{172}.

Patients with active SLE have high concentration of circulating TNF-\(\alpha\) in circulation and in kidney biopsies\textsuperscript{163,168}, levels drop down to normal during periods of remission. \textit{Fas} deficient animals also exhibit high levels of TNF-\(\alpha\)\textsuperscript{128}. Surprisingly, treatment of NZB/W F1 animals with TNF-\(\alpha\), when started at 14 weeks, resulted in disease amelioration, whereas such a beneficial effect was not seen when treatment was initiated at twenty seven weeks\textsuperscript{173}. The cytokine may thus have contrasting effects, depending on the stage of disease.
Interferon-α appears to be a key cytokine in SLE pathogenesis as high levels of this cytokine are frequently encountered. When IFN-α is administered therapeutically for other conditions, auto-antibodies typical of SLE appear\textsuperscript{174}. Elevated levels of the cytokine are also observed in C4 knockout mice\textsuperscript{134}. The presence of IFN-α in young mice before any signs of disease suggests that the activation of IFN-α system in SLE is a prerequisite rather than a result of disease. Upon microarray analysis of the PBMCs from SLE patients, it was observed that fifteen genes were up-regulated, out of which fourteen were targets of IFN-α. Analysis of PBMCs from a patient in remission demonstrated no significant differences in gene expression from controls\textsuperscript{175}, indicating relevance of the observations to active disease. Anti-dsDNA and DNA-containing immune complexes and/or apoptotic bodies have been shown to induce the generation IFN-α from plasmacytoid dendritic cells, resulting in the maturation of monocytes to dendritic cells. Such phenomena may constitute major contributing factors in lupus pathology\textsuperscript{176}.

**Genetic involvement**

SLE rarely occurs as a single gene defect. It is a polygenic trait in which a number of MHC and non-MHC genes interact with environmental factors to create varied disease phenotypes. More than fifty loci have been found to affect the susceptibility to murine lupus. These loci are dispersed on chromosomes one, four, seven, ten and seventeen\textsuperscript{177}.

In genetic mapping studies in MRL\textsuperscript{1pr/1pr} mice, four loci were found to have varying degrees of influence on lupus predisposition\textsuperscript{178}. \textit{Lmb1} congenic animals had enlarged spleen and lymph nodes. \textit{Lmb2} and \textit{Lmb4} loci caused slightly larger lymph nodes but enhanced proliferation in the spleen was not observed. \textit{Lmb4} appeared to be involved in the induction of glomerulonephritis at the late stages of disease, in addition to aiding in lymphoproliferation. \textit{Lmb3}, which was mapped to a 0.9 Mb interval\textsuperscript{179} of the chromosome seven, is believed to have a major influence on lymphoproliferation and the induction of anti-chromatin antibodies.

Other important loci are \textit{sle1, sle2, sle3} on chromosomes one, four and seven respectively. \textit{Sle1} by itself is sufficient to generate high-titre antibody response to dsDNA, chromatin and histones (H2A/H2B/DNA complex). It is strongly associated with glomerulonephritis and induces a high serum concentration of IgG. The T cells demonstrate proliferative responses to histones in \textit{sle1}
congenic animals. When this locus was further divided into 4 loci- sle1a, sle1b, sle1c, sle1d, it was found that sle1a and sle1b are the most potent loci, each being capable of mediating fatal lupus when combined with yaa or lpr. There was a significant increase in the levels mRNA and protein of IL-6 in these animals.

Sle2 is associated with B cell hyperactivity, polyclonal B cell activation and an increase in the B-1a subset of B cells (with a phenotype of IgM^+ B220^{low} CD5^+ CD23^- CD43^+ CD11b^-)182. These cells are the major source of serum IgM and positive selection by auto-antigens plays an important role in their development. The expansion in the B-1a compartment is due to increased proliferation, decreased apoptosis and increased cell production by the fetal liver. This locus is further subdivided into Sle2a, Sle2b and Sle2c. Even though Sle2c contributes to an increase in B-1a cells, it does not appear to accelerate lupus pathogenesis. Sle2a and Sle2b have been shown to increase the lymphocytic expansion and kidney pathology. A combination of Sle2a with Sle1 and Sle3 resulted in significantly higher proteinuria than when only Sle1 and Sle3 where present.

Sle3 is responsible for increased serum levels of IgM and IgG antibodies, causing immune complex-mediated glomerulonephritis, lymphadenopathy and anti-dsDNA antibody production. Expression of CD69 on T cells increased with age, indicating an activated phenotype. On stimulation with anti-CD3 and anti-CD28, T cells from congenic animals showed significantly enhanced proliferation, accompanied by a reduction in the apoptosis of activated T cells184. A number of genes identified as contributing to lupus susceptibility in mouse models lie in close vicinity of this locus.

Other loci such as Sbw1 and Sbw2 also contribute to splenomegaly, glomerulonephritis and mortality.

In humans, no associations have been found with the MHC I locus, though there is evidence implicating the MHC II region. DR-B1 alleles, DR2 and DR3 have been consistently found to be associated with disease in European-Caucasian lupus patients and DR and DQ alleles show a stronger association with the presence of auto-antibodies like anti-Ro and anti-La. In African-American patients, HLA-DR2 and HLA-DR7 have been associated with SLE.
Therapies
The first therapies, initiated in the 1950s, consisted of corticosteroids, immunosuppressive agents like cyclosporin and anti-malarial medicines. Hydroxychloroquine for the skin and joint manifestations has since then been successfully used. It works by interfering with antigen processing, inhibiting phagocytosis and neutrophil migration. Corticosteroids have anti-inflammatory and immunosuppressive effects, but their use is usually associated with multiple side-effects, necessitating the use of other medications. The efficacy of the combination of cyclophosphamide and prednisolone has been reported. Additionally, intravenous immunoglobulins (IVIG) have also been successfully used. Drugs such as mycophenolate mofetil, which suppresses T and B cell proliferation, have been shown to be efficacious in the lupus mouse models and in human patients, and fewer side effects have been reported.

B cells are known to play a key role in lupus. Thus, it was hypothesized that B cell removal would lead to lupus amelioration. Rituximab is a chimeric monoclonal antibody reagent consisting of human IgG1 and kappa constant regions from a murine hybridoma directed at human CD20. Treatment with this molecule results in almost complete depletion of B cells from the periphery, an effect that lasts for more than 6 months. Within one week of treatment, Rituximab causes down-regulation of co-stimulatory molecules CD40 and CD80 on the CD19+ B cells. Rituximab does not however, lower the numbers of plasma cells, as they do not express CD20. Even after B cell numbers in the circulation return to normal levels after treatment, patients remain in remission.

Hemoglobin
Hemoglobin (Hb) is the most extensively studied protein in vertebrates. It is always enclosed the red blood cells, and its major function is to transport oxygen throughout the body. The quaternary structure of Hb is composed of two alpha and two beta subunits which are very similar to each other. Both subunits have high structural homology to myoglobin, a monomeric oxygen-binding protein present in the muscles. Each subunit of Hb is associated with heme (which is protoporphyrin IX) with an iron atom in the centre (Figure II a); it is the presence of heme that gives Hb its red colour. Normally, the iron atom is always in the ferrous oxidation state, whether it is bound to oxygen or not. The heme moiety is present in other proteins as well, such as catalase, the cytochromes and peroxidase. Heme has a planar structure. Fe$^{2+}$ binds to the four porphyrin
rings through coordinate bonds (Figure II b). The fifth coordination bond which is below the plane, is to the histidine side chain of the globin moiety of the protein. When heme is bound to oxygen, it forms the sixth coordination bond above the plane.

Figure II: a) Hemoglobin structure. b) Planar structure of heme, showing 4 coordination bonds to nitrogen (N) of four porphyrin rings (labeled A to D) and one to nitrogen (N) of histidine side chain of the protein (below the plane). The sixth coordination bond is to the oxygen molecule that it carries.

In RBCs, a small percentage of Hb is always oxidized from the ferrous (Fe$^{2+}$) form to ferric (Fe$^{3+}$) form, resulting in the formation of methemoglobin (metHb). MetHb cannot bind oxygen as it is octahedrally coordinated to a water molecule. Erythrocytes contain methemoglobin reductase which functions to reduce metHb$^{190,191}$.

When Hb is released in the plasma due to intravascular lysis of RBCs, the quaternary structure breaks down into dimers$^{192}$ which bind Haptoglobin (Hp), an acute phase protein$^{193}$. Hp is a serum glycoprotein that binds Hb by a strong, irreversible, non-covalent bond with dissociation constant being $10^{-15}$ moles/litre$^{194}$. It is synthesized as a single polypeptide that is cleaved proteolytically.
into a smaller alpha chain of around 10 KDa and longer beta chain of mass around 39 KDa. These then connect through disulphide bonds\textsuperscript{195}. Binding of Hb to Hp results in stabilization of the former, preventing the release of toxic heme in the circulation (see below).

Formation of Hb-Hp complex exposes a neo-epitope, permitting high affinity binding to the CD163 molecule, a scavenger receptor, with a molecular mass of 130 KDa\textsuperscript{196}. The Hb-Hp complex is taken up and degraded, mainly in the liver and the spleen; recent evidence indicates the presence of CD163 in circulating monocytes as well\textsuperscript{197}. Binding is believed to induce intracellular signaling and mediate anti-inflammatory responses. Soluble CD163, formed upon proteolytic cleavage under inflammatory conditions has been found to be high in the patients suffering from rheumatoid arthritis\textsuperscript{198}. CD163 also acts as immuno-modulator, because clearance of Hb results in the formation of carbon monoxide which is anti-inflammatory (see below). Additionally, CD163 binding results in increased secretion of IL-6 and IL-10\textsuperscript{196}.

If, upon release into circulation, Hb is not bound by Hp (under disease conditions discussed below), free heme is released. Heme binds to hemopexin (Hx) with a very high binding capacity (K\textsubscript{d} being less than 1 pM). The Heme-Hx complex is then degraded in the liver. Uncomplexed heme can intercalate into the lipid membranes of cells as it is very lipophilic\textsuperscript{199}. Intracellularly, heme is broken down to iron, biliverdin and carbon monoxide via the action of heme-oxygenase I. Ferritin synthesis is also up-modulated; the protein serves to sequester free iron and so protects the cell from the ROS-mediated damage that may result due its presence. Ferritin is multimeric protein composed of twenty four subunits of two types – a heavy chain and a light chain, and demonstrates a very high binding capacity for iron (4500 moles of iron per mole of Ferritin)\textsuperscript{199,200}.

Under conditions of extensive RBC lysis as malaria, sickle cell anemia and autoimmune hemolytic anemia, Hp levels decrease below detectable levels. Low levels of unconjugated hemopexin levels have also been reported\textsuperscript{192,201,202,203}. Excessive hemolysis therefore saturates and overwhelms these Hb removal systems and leads to build up of Hb (to concentrations in the range of 20-150 \(\mu\)M)\textsuperscript{204} and heme in plasma\textsuperscript{192} (Figure III).
Ferrous Hb has very high affinity for Nitrous Oxide (NO), which acts as a muscle relaxant; removal of this gas by free Hb causes vascular constriction\textsuperscript{205} and endothelial dysfunction such as disruption of smooth muscle tone and platelet activation\textsuperscript{192}. By virtue of direct contact with the bloodstream, endothelial cells are the first target of Hb-mediated damage\textsuperscript{206}. Hb induces caspase-mediated apoptosis in cultured endothelial cells\textsuperscript{207}. Additionally, free Hb disrupts the endothelial cell integrity via oxidative modification of the plasma membrane\textsuperscript{208}.

These observations may be of special relevance to lupus. As indicated above, free Hb in circulation (arising because of circulating anti-RBC antibodies) rapidly breaks down to dimers\textsuperscript{209} which could be oxidized in the presence of ROS\textsuperscript{192} (a condition that characterizes the inflammatory environment seen in SLE\textsuperscript{210,211}). The oxidized dimers then break down to release free heme, and globin (as shown in Figure III). As indicated, free heme is extremely toxic as it can easily enter the cells because of its lipophillic nature. In cells, the release of free iron causes oxidative damage by generation of other ROS by Fenton reaction\textsuperscript{212}; lipid peroxidation may also occur\textsuperscript{213}. Hb dimers and heme are also avidly taken up by the kidneys resulting in nephrotoxicity\textsuperscript{211,214}.

Hb causes brain injury, both by itself and its degradation products; brain edema results within twenty-four hrs of exposure. Hb acts by inhibiting the Na\textsuperscript{+}/K\textsuperscript{+} adenosine triphosphate activity,
causes lipid peroxidation\textsuperscript{215}, brain swelling, increase in brain water content, excitotoxic death of neurons in the cortex and induces depolarization of hippocampal neurons\textsuperscript{216}. Terminal deoxynucleotidyl transferase-mediated dUTP nick end-libeling (TUNEL)-positive neurons and astrocytes are found around the area of hemorrhage in the brain\textsuperscript{217}. When primary neurons are exposed to Hb, time and dose dependent cytotoxicity is observed.

Some evidence of the antigenicity and immunogenicity of Hb exists. Despite the high degree of homology in the amino acid sequences of Hb of different species, xeno-immunization leads to the production of specific antibodies. Five major B cell epitopes have shown to be present on both alpha and beta subunits of human Hb\textsuperscript{218,219}. Antibody reactivity against human Hb has been reported in patients of sickle cell anemia, possibly related to repeated transfusion\textsuperscript{220}; antibodies target the mutation site. Aberrant T cell reactivity to the Hb-Hp complex has been reported in the NZB animals that have extensive hemolytic anemia. Autologous RBC antigens appeared to be a stimulus for CD4\textsuperscript{+} T cell proliferative responses\textsuperscript{221}. 