Chapter 2

Pathogenesis of Lupus: Complex Interplay of Immunological Factors

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INTRODUCTION

Systemic lupus erythematosus (SLE) is an enigma to both the treating physician and the scientist attempting to decipher the pathogenesis. The disease is thought to be a result of imbalance between the effector and regulatory arms of the immune system. The pathogenesis of SLE is complex with multiple players contributing to it. Lupus pathogenesis is a multistep process with genetic susceptibility to start with followed by a phase of preclinical lupus and then clinically manifest disease.This review presents the current evidence in a logical sequence to describe the interplay of immunological factors in the pathogenesis of lupus (Fig. 2.1).

GENETIC FACTORS

The role of genes in predisposing to SLE is reflected by twin and family studies. With a heritability of up to 43.9%, higher incidence in monozygotic twins (25–46%), a 5.87% relative risk among the first-degree relatives and a high-risk ratio of up to 29% in the siblings of SLE patients support the role of genes in disease pathogenesis. Further, the genes also modulate end organ involvement and severity of clinical manifestations in SLE.[1] Genes in the major histocompatibility complex (MHC) region play a major role in SLE susceptibility across various populations. With the advent of Genome Wide Association Studies (GWAS) numerous non MHC genes have also been implicated with SLE. A brief account of various HLA and non-HLA genes associated with SLE across various populations is represented in Tables 2.1 and 2.2.[2] Association of HLA with antibodies in SLE has been shown in Table 2.3 and the role of selected few genes are discussed later in the course of this review.

ENVIRONMENTAL FACTORS

Environmental exposure to various agents is the most important inciting event in initiating autoimmunity. Though epidemiological evidence for all the proposed toxins and infections are not convincing, *in vitro* data and animal models support such cause effect relationships (Table 2.4).[3]

production in genetically predisposed patients.[9] Over all, the current evidence supports a model where in initial environmental trigger upregulates IFN-γ, Th1 and Th2 cytokines, which drives IFN-α production, B cell activation, ultimately resulting in variable autoimmunity.

STAGE OF EARLY AUTOANTIBODY FORMATION

The autoantibodies in SLE appear approximately 9.4 years (mean 3.3 years) prior to clinical signs or symptoms. Various autoantibodies starting with anti-nuclear antibody (ANA) and anti-Ro progressing to anti-cardiolipin (aCL) and anti-Smith (Sm) appear at different time frames during the course of immune dysregulation. The order of appearance of these antibodies have no bearing on the outcome and prognosis of SLE. Current evidence suggests that a marked rise in the titer of anti-dsDNA antibodies may herald the onset of SLE in asymptomatic individuals.[3]

PROGRESS OF AUTOANTIBODIES: FROM BENIGN TO PATHOGENIC

The autoantibodies seen in SLE are generated against various cellular components like DNA, DNA binding proteins, RNA, RNA-binding proteins, ribonuclear proteins and phospholipids in the cell membrane. The primary autoantibodies generated are of IgM subclass which are either pathogenic or protective. Some of the natural IgM autoantibodies have anti-apoptotic, and inhibitory effect on TLR mediated cell activation.[10] However, as the immune dysregulation progresses, the autoantibodies undergo class switch to IgG, IgA or IgE subtype and escape into the intravascular or mucosal surface and get deposited in various organs.

Some of the ANAs also act as DAMPs and upregulates inflammatory pathways. Antibody production in SLE is further enhanced by elevated IL-21 and T follicular (Tfh) cell activation. In addition, excessive B cell stimulation by antigen primed CD4+ helper cells and B cell activating factors (BAFF and APRIL) also increases antibody production. The specific autoantibodies in SLE like the anti-Sm and anti-dsDNA are used as markers of diagnosis. Autoantibodies against C1q, dsDNA, anti-Ribosomal P play important roles in the pathogenesis by getting deposited in renal and neural tissues[11] leading to amplification of inflammatory cascade.

ROLE OF DEFECTIVE APOPTOSIS, COMPLEMENTS, CELL DEBRIS CLEARANCE AND PAMPS

Various factors including toxins, infections, physical and psychological stress lead to increased apoptosis of cells which in normal individuals are effectively cleared by phagocytes. Several GWAS studies in SLE have shown strong association with genes affecting the receptors responsible for clearance of cell debris and immune complexes (FCGR2A, FCGR2B, FCGR3B, ATG5, CLEC16A). This is further compounded by an intrinsic defect in macrophages to effectively phagocytose cell debris in SLE.

Clearance of immune complexes and debris is naturally aided via coating of apoptotic cells with C1q complement component, C-reactive proteins (CRP) or serum amyloid P (SAP).[12,13] Low complement levels, defective complement receptors and antibodies to complement components (e.g. anti-C1q) result in poor immune complex handling and thus favours deposition in tissues. These defects also lead to persistence of the self and foreign antigens resulting in excessive DAMP-mediated toll-like receptors (TLR) stimulation.[14]

DC are responsible for effective clearance of apoptotic debris by the means of various receptors. This step is compromised in patients prone to develop lupus leading to prolonged self-antigen exposure, resulting in disruption of immune tolerance. The pDCs are a major source of type I IFN which plays an important role in initiation and amplification of immune dysregulation.[23]

T Cells

All the main T cell subsets (CD4+, CD8+, and double negative T cells) are aberrantly activated in SLE. This results in inappropriate cytokine release and excessive activation of B cells. Several alterations are noted in the T cell receptors (TCRs) and the downstream signaling of these cells in patients with lupus (Fig. 2.5).

T follicular helper cells (Tfh) are required for immunoglobulin class switching, formation of memory B cells and determining the clonality of B cells with the help of IL-21. The Tfh cells express CXCR5 which allows them to localise to B cell zone in the follicle. Their functions are regulated by the expression of programmed cell death protein 1 (PD-1) which is a negative regulator, the inducible T-cell co-stimulator (ICOS) and the OX40 ligand which are positive regulators. Studies involving lupus mice models have demonstrated overexpression of ICOS and OX 40 on the Tfh cells. In human studies,Tfh cells are increased both in the tertiary lymphoid organs (TLOs) and in circulation (cTfh). The role played by Tfh in the pathogenesis of SLE and the various factors influencing Tfh activation is represented in Fig. 2.5.[24]

Th17 cells are emerging as novel players in lupus pathogenesis. Th 17 cells are activated by IL-23 and they produce various IL-17 cytokines (IL-17A to IL-17F). The level of these cytokines, mainly the IL17A corelates with disease activity in lupus. Studies from lupus mouse models and humans have demonstrated an increase in the numbers of Th17 in patients with SLE as compared to healthy controls.[25] These cells are also found in tissue biopsies of kidneys in LN, reflecting a local as well as a systemic role in immune dysregulation. Activation of the Th17 cells tilts the balance of Th17/T-reg towards a Th17 predominant inflammatory phenotype.[26]

B Cells

The production of autoantibodies, mainly against the self-nuclear antigens represents the loss of tolerance in B cells. B cell related gene (BLK, BANK and PTPN22) polymorphism have been associated with the development of aberrant response to autoantigens. Beyond antibody production B cells also double up as antigen presenting cells and a source of inflammatory cytokines. The B lymphocytes are activated by different cells including the Th1, Tfh, and are affected by NETosis (Figs 2.3–2.5). B-cells can also activate the TLRs on the innate cells by producing high affinity antibodies against chromatin materials resulting in an antigen antibody complex. These complexes also activates the classical complement pathway, resulting in damage of tissues and further precipitation of NETosis. Autoantibody subclasses IgG and IgM are usually implicated in the pathogenesis of SLE. Recent evidence however has demonstrated a high level of IgE and IgE subclass of autoantibodies along with activated basophils in patients with lupus.[27] Experiments in mouse models have demonstrated a beneficial effect of IgE depletion in treating renal disease, thus throwing open a new vista for possible experimental therapeutic intervention.[28] Several therapeutic trials with rituximab (anti-CD20), belimumab (anti-BAFF) and tabalumab (anti-APRIL) though not completely successful have indirectly demonstrated the role of B cells in the pathophysiology of SLE.[29]

Table 3.1: Different patterns of anti-nuclear antibodies seen on IIF assay

Pattern	*Target antigens*
Homogeneous	DNA, histones, nucleosome
Speckled	
Fine	Ro (SS-A), La (SS-B)
Coarse	Sm, nRNP
Centromere	Centromeric protein A, B
Nucleolar	RNA polymerase, PM-Scl
Envelope	Laminins

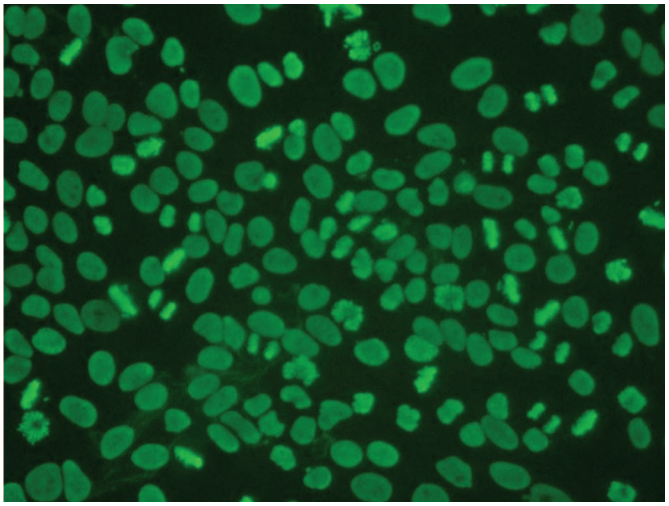

Fig. 3.1: Homogenous ANA pattern as seen on Hep2 cells using indirect immunofluorescence assay

only increases the likelihood of a diagnosis of connective tissue disease like SLE in an appropriate clinical setting.

If ANA is negative and clinical suspicion of SLE is high then anti-Ro antibodies, anti-phospholipid antibodies should be tested as they can be present in SLE patients. Further chronic renal insufficiency and use of B cell depletion therapies can also result in negative ANA in a patient with SLE.[3]

The higher the titer the more likely it is of clinical significance. Once the ANA is positive, sub-specificity testing can help in disease classification and prognostication. Presence of antibodies to dsDNA have a strong association with nephritis.

However, there is no need to repeat ANA as it stays positive throughout the life of an SLE patient and most therapies do not significantly affect its levels except B cell depletion therapy.

ANTIBODIES TO dsDNA

In the nucleus the double strand of DNA is wound around histone proteins. The antibodies can be formed against dsDNA, histones or a complex of dsDNA/histone. The complex of 100bp nucleotides along with histones are called nucleosome.[4] Antibody levels can be