

Murine Lupus: Pathogenic Mechanisms and Genetic Origins

C Mohan,*^{MBBS, PhD}

Abstract

Research in several murine models of lupus, reinforced with patient studies, has served to identify key events leading to lupus nephritis. Among these, anti-nuclear antibody (ANA) induced end-organ damage, production of such ANAs by intrinsically hyperactive B-cells, and antigen-specific, cognate T-cell help have been well documented. Though studies in parallel fields of immunology have uncovered a plethora of molecular defects that can potentially drive these key pathogenic events, the actual molecular defects that are responsible for these events in spontaneous lupus remain unknown. Whereas genetically pre-determined triggers are most likely responsible for each of these pathogenic events, humoral and environmental triggers are likely to exert secondary effects, in attenuating or accentuating these mechanisms. Recent genetic studies in mice have allowed researchers to dissect this complex autoimmune disease into a collection of simpler immunological aberrations. These simpler murine models will facilitate the identification and characterization of the fundamental building blocks of lupus, and advance our understanding of the genetics and pathology of this intriguing disease.

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Introduction

The complex and non organ-specific nature of systemic lupus erythematosus (SLE) has made it difficult for researchers to unravel the genetic defects and pathogenic mechanisms underlying this disease. Over the past 30 years, several mouse models of lupus bearing differing sets of phenotypes and genotypes, have collectively contributed a great deal towards our understanding of the disease, as recently reviewed.^{1,2} Key pathogenic events are highlighted in Figure 1, and are further described below.

The Pathogenic Role of Anti-nuclear Antibodies

The emergence of anti-nuclear antibodies (ANAs) is the hallmark of lupus, and perhaps represents the final common effector pathway, shared by the different murine lupus models and SLE patients, through which various causative mechanisms may act to induce renal damage. The detection of these antibodies by a variety of laboratory methods remains the cornerstone of clinical diagnosis.³⁻⁸ Indeed, numerous clinical studies have underlined the correlation between serum ANA levels and disease progression.^{8,9} Pathogenicity of these antibodies has also been directly shown by transfer into normal or pre-autoimmune mice.¹⁰ However, not all ANAs are pathogenic. Several features, including their cationic charge,

isotype, fine-specificity and cryoprecipitability, determine their pathogenic potential.^{11,12} ANAs that bear the right physicochemical properties may induce end-organ damage by (i) targeting directly to preplanted or in-

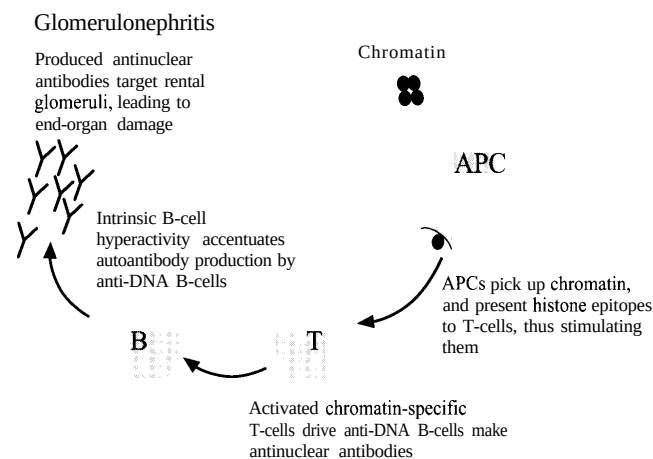


Fig. 1. Multiple pathogenic events lead to nephritis in lupus. Presentation of self-chromatin by antigen-presenting cells (APCs) in an immunogenic fashion is likely to trigger loss of T-cell tolerance to this antigen. Since anti-DNA B-cells can also pick up chromatin with high efficiency, and present the relevant histone epitopes to the T-cells, they can also be driven by the activated chromatin-specific T-cells, to secrete ANAs. The intrinsic hyperactivity of these B-cells may serve to further augment autoantibody production. The produced ANAs are pathogenic, in that they can get deposited in the different end-organs, including the kidneys, trigger the inflammatory cascade, and precipitate the clinical manifestations of lupus.

* Research Scientist

Pathology, Immunology and Laboratory Medicine & Center for Mammalian Genetics
University of Florida, USA

Address for Reprints: Dr Chandra Mohan, Pathology, Immunology & Laboratory Medicine, JHMH, Box 100275, University of Florida, Gainesville, Florida 32610-0275, USA.

situ antigens, such as DNA, histones, nuclei, or components of glomerular basement membrane such as laminin and heparan sulphate, or (ii) by first forming immune-complexes with nucleosomes, then getting deposited onto anionic sites on glomerular basement membrane, via histone bridges.¹³⁻²⁰ In addition, both murine and patient studies have also revealed that non anti-DNA antibodies may also have a pathogenic role in this disease.^{12,20} In addition, several secondary factors determining immune-complex clearance rates (which can potentially be compromised by abnormalities in FcR, complement receptors, complement, and C-reactive protein), local cytokine profiles in end-organs (e.g. transforming growth factor β 1, tumour necrosis factor and interleukin-1), and the differential expression of target antigens in the kidneys, may all serve to modulate the degree of pathogenicity of these autoantibodies, as previously discussed.¹ Importantly, genetic studies and murine knockouts have demonstrated the potential contribution of some of these molecules, notably the complement proteins, and the cytokine, transforming growth factor β 1, to lupus pathogenesis.²¹⁻²⁴

Intrinsic B-cell Hyperactivity may be a Common Potentiator

Numerous murine studies showing polyclonal seroreactivity, intrinsic B-cell hyper-responsiveness to lipopolysaccharide (LPS) and conventional antigens, and heightened, spontaneous B-cell proliferation and IgM secretion *in vitro*, elegant cell-transfer experiments, and corroborating studies in human lupus all point to intrinsic B-cell hyperactivity as one of the common denominators underlying both murine and human lupus.²⁵⁻³⁹ Transgenic and knockout experiments in parallel fields of immunology have demonstrated how global defects in B-cell tolerance (as exemplified by defects in FAS, FasL and bcl-2), and defects in key signaling molecules (such as Shp1, lyn, Cr2, vav, btk, CD19 and CD22), can both modulate B-cell hyperactivity and autoantibody generation.^{1,2} Moreover, the aberrant expression of interleukins impacting B-cell function, especially interleukin-6 and interleukin-10, have also been strongly implicated in lupus pathogenesis.⁴⁰⁻⁴² Nevertheless, the actual genetic defects underlying B-cell hyperactivity in murine and human lupus remain a mystery. However, genetic studies of murine lupus stress that though B-cell hyperactivity may contribute to ANA formation, it is not necessary, nor sufficient for the eventual development of lupus nephritis;⁴³⁻⁴⁵ it may simply serve to amplify the ongoing autoimmune response.

Antigen-specific Helper T-cells Drive Lupus B-cells make ANAs

The oligospecificity and structural features of pathogenic ANAs provide strong evidence that some addi-

tional factors, extrinsic to generalized, polyclonal B-cell activation, are crucial in driving the expansion of autoimmune B-cells. The concomitant presence of anti-histone and anti-histone/DNA antibodies in patients with anti-DNA antibodies, specific for conformational DNA-protein epitopes dependent on the quaternary structure of chromatin, suggests that chromatin may be the culprit immunogen in this disease.⁴⁶ Anti-chromatin ANAs in human and murine lupus evolve in a step-wise manner, being directed initially against conformational epitopes on native chromatin and the (H2A-H2B)₂-DNA subnucleosome, with subsequent appearance of anti-histone and anti-dsDNA specificities.⁴⁶⁻⁴⁹ The kinetics of appearance of these ANAs, their IgG2a/IgG2b subclass preference, and the increased expression of arginine replacement mutations in their complementarity determining regions^{8,50} are all consistent with being the outcome of T-dependent immunization with chromatin. Parallel studies involving neonatal thymectomy, nude mutations, and anti-CD4 treated mice have clearly underlined the role of CD4+ T-cells in the pathogenesis of lupus?⁵¹ CD4+ T-cells have been shown to drive lupus B-cells to selectively secrete anti-DNA ANAs, through cognate, contact-dependent interactions, in murine lupus,⁵⁴⁻⁵⁸ as well as in lupus patients.⁵⁹⁻⁶¹ Importantly, these Th cells have been shown to be pathogenic *in vivo*,⁶² and may be specific for chromatin and other DNA-binding proteins.⁶³⁻⁶⁷

Several immunological events have been shown to have the potential to contribute to this pathogenic mechanism. The *lpr* mutation of the FAS molecule demonstrates how global defects in peripheral T-cell tolerance can accentuate this pathogenic step.^{68,69} In addition, aberrant activation of anti-chromatin T-cells by cross-reactive environmental/viral antigens, as discussed below, may also trigger this pathogenic event. Though skewed Th1/Th2 interleukin profiles have also been shown to have pathogenic consequences in this disease, it is now clear that both Th1 (such as interferon γ) and Th2 (such as interleukin 4) cytokines can, and do, contribute to the pathogenesis of lupus.^{1,70-72} Despite these insights, the actual molecular defects responsible for breaking T-cell tolerance to chromatin in murine or human lupus remain to be deciphered.

The different stages/steps in the pathogenesis of lupus outlined above are pictured in Figure 1. ANA-induced glomerulonephritis remains the most prevalent cause of pathology underlying lupus. Such pathogenic ANAs appear to arise from B-cells that are intrinsically hyperactive and/or driven into expansion by helper T-cells in an antigen-specific manner. Though parallel advances in other branches of immunology have highlighted a wide spectrum of molecules that can potentially modulate T-helper activity, B-cell ANA formation, and ANA-induced pathology in lupus, the precise mo-

lecular defects that are responsible for these individual pathogenic mechanisms in spontaneous lupus await genetic dissection.

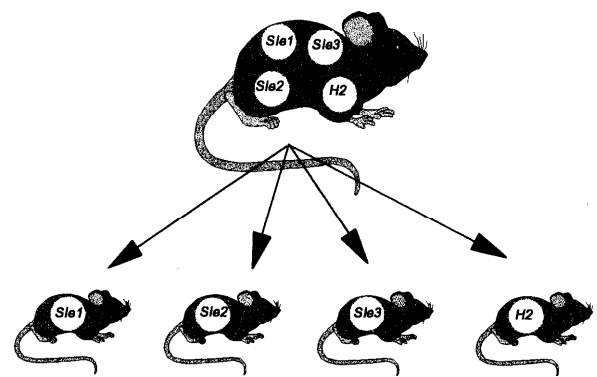
Genetic Dissection of Pathogenic Mechanisms Leading to Lupus

Our recent studies have focused on a genetic approach to dissecting the component phenotypes of SLE, in order to explore each underlying pathogenic mechanism. Recent advances in molecular genetics have led to the development of interval mapping techniques employing extensive sets of highly polymorphic simple sequence repeats as genetic markers.^{73,74} Using this elegant technique, several groups have recently identified distinct chromosomal intervals that confer susceptibility to murine lupus. Among these, *Sle1* on murine chromosome 1 (most closely linked to D1MIT15), *Sle2* on murine chromosome 4 (most closely linked to D4MIT9), *Sle3* on chromosome 7 (most closely linked to the *p* locus), and the *H2* (MHC) locus on chromosome 17, correlate strongly with lupus susceptibility.⁷⁵⁻⁷⁹

To facilitate the identification of the culprit genes within these intervals, and to fathom each locus' role in disease pathogenesis, each of these individual intervals has now been successfully back-crossed onto the normal C57BL/6 (B6) background.⁸⁰ Importantly, these congenic strains bear the "normal" B6 alleles at all other loci, except at the selected chromosomal intervals, where they express the "abnormal" allele(s) derived from a lupus-prone, NZM2410 strain. Systematic phenotyping of these congenic strains (along with B6 controls) now allows us to dissect out the immunological effects of each of these lupus susceptibility loci.

Interestingly, each of these congenic strains, B6.NZMc1 mice bearing *Sle1*, B6.NZMc4 mice bearing *Sle2*, and B6.NZMc7 mice bearing *Sle3*, express different component lupus phenotypes present in the parental NZM2410 lupus strain.^{45,81-83} As summarized in Figure 2, *Sle1*, by itself, on a normal B6 background, spontaneously leads to high levels of IgG anti-chromatin antibodies and chromatin-specific T-cells, with little evidence of nephritis.^{82,83} Interestingly, the syntenic chromosome 1 interval has also been recently demonstrated to be linked to ANA generation in human SLE.⁸⁴ In contrast, *Sle2* leads to elevated serum levels of polyclonal/ polyreactive IgM, hyperactive B-cells, and an expanded population of B1-cells.⁴⁵ We believe that the B-cell hyperactivity induced by *Sle2* might serve to amplify an ongoing autoimmune response. *Sle3* bearing mice exhibit elevated serum polyclonal/ polyreactive IgM and IgG, increased numbers of activated helper T-cells, and also glomerulonephritis.⁸² Ongoing studies explore how these different susceptibility loci collaborate to precipitate and accentuate autoimmune pathology. As we review the body of data that Figures 1 and 2 represent, we are

NZM2410 lupus strain



Congenic strain	B6.NZMc1	B6.NZMc4	B6.NZMc7	B6.H2z
Polyclonal activation of B-cells	No	Yes	Yes	No
Selective loss of tolerance to chromatin	Yes	No	No	No
Glomerulonephritis	No	No	Yes	No

Fig. 2. Loci on chromosomes 1, 4, 7 and 17 confer susceptibility to lupus in the NZM2410 strain.⁷⁵ When these different susceptibility intervals are individually bred onto the normal C57BL/6 background, these "congenic" mice display different component phenotypes that constitute lupus.^{45,82,83} B6.NZMc1 mice bearing *Sle1* on a normal B6 background, spontaneously leads to high levels of IgG anti-chromatin antibodies and chromatin-specific T-cells, with little evidence of nephritis. In contrast, *Sle2* leads to elevated serum levels of polyclonal/ polyreactive IgM, hyperactive B-cells, and an expanded population of B1-cells. *Sle3* bearing mice exhibit elevated serum polyclonal/ polyreactive IgM and IgG, increased numbers of activated helper T-cells, and also glomerulonephritis. These "congenic" strains now allow us to study each of the relevant pathogenic mechanisms in greater detail.

beginning to appreciate which lupus-susceptibility locus contributes to which specific pathogenic mechanism(s), in order to orchestrate the disease.

A challenge that lies ahead is to identify the genes responsible for human lupus. One approach as adopted by Tsao and colleagues,⁸⁴ is to extrapolate directly from these murine studies, i.e. to screen microsatellite markers in the syntenic human intervals in lupus-afflicted sib-pairs, in order to uncover any significant linkage. An alternative, and more comprehensive approach is to carry out microsatellite-based genome-wide scans of lupus-afflicted sib-pairs or multiplex families, in order to locate disease-susceptibility intervals, which may or may not overlap the murine loci. Together, these genetic explorations of murine and human lupus promise to enrich our understanding of how individual immunological aberrations can build up, to orchestrate lupus.

Secondary Factors: Hormones and Environment

Studies in several laboratories have clearly shown that the female predominance seen in lupus and other systemic autoimmune diseases is not due to sex-linked genes, but due solely to hormonal effects: androgens

suppress, whereas oestrogens accelerate disease.⁸⁵ With respect to the key pathogenic steps outlined above, it appears that these hormones can potentially exert their effects by tuning B-cell or T-cell function, directly. Earlier reviews have detailed how females have more exuberant antibody responses when challenged with exogenous antigens.^{85,86} Sex hormones may impact B-cell function by modulating the production of interleukins 4, 5, or 6, by impacting the function of B1-cells, or yet other mechanisms.⁸⁷⁻⁸⁹ Available data suggest that these hormones might impact T-cell function by primarily impacting cytokine production (especially interleukin-2 and interferon- γ), and Th1/Th2 balance.⁹⁰⁻⁹³ Besides these direct impacts on lymphocyte function, it is not clear if hormones have the potential to affect autoantibody-mediated end-organ damage.

Murine models that develop lupus, do so with a penetrance ranging from 80% to 95%, independent of the geographic breeding location. In contrast, the concordance rate in human SLE in monozygotic twins is about 57%.⁹⁴ Considering these facts in light of the observation that lupus is a polygenic disease, it is reasonable to propose that if a given individual or strain carries a sufficient genetic load of susceptibility genes, all the pathogenic events outlined above can progress maximally, until autoantibody-induced end-organ damage ensues. On the other hand, in the absence of the full complement of disease genes, environmental or hormonal effects might play a significant role in accelerating any "rate-limiting" pathogenic mechanisms. How immune responses to viral and microbial pathogens can contribute to autoimmunity has recently been reviewed.⁹⁵ Briefly, viral or bacterial infections have been reported to contribute to lupus through a variety of mechanisms, including the breaking of T-cell tolerance to self-chromatin,⁹⁶ polyclonally stimulating B-cells,^{97,98} or by triggering interferon- γ production and Th1/Th2 skewing.⁹⁹ Finally, among the better studied dietary factors known to impact lupus pathogenesis, these seem to be contributing to disease, primarily by affecting cytokine production.¹⁰⁰

Conclusions

Immunological investigations of lupus demonstrate how several distinct mechanisms impacting B-cells and T-cells collaborate, to generate high titres of pathogenic anti-nuclear antibodies, which are largely responsible for pathology in this disease. Whereas genetically predetermined triggers might be primarily responsible for each of these pathogenic events, humoral and environmental triggers are likely to exert secondary effects, in attenuating or accentuating these mechanisms. Recent genetic advances now allow researchers to dissect this complex autoimmune disease into a collection of simpler immunological aberrations. We believe these sim-

pler murine models will not only serve to identify and characterize the fundamental building blocks of lupus, but also the culprit genes, eventually.

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