

Delineating the Genetic Basis of Systemic Lupus Erythematosus

Review

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Summary

Genetic predisposition plays a crucial role in susceptibility to systemic lupus erythematosus (SLE) in both human patients and animal models. Recent progress in experimental systems and human linkage analysis is providing key insights into the genetic basis for susceptibility and elucidating the manner in which genetic interactions mediate severe disease pathogenesis. Genes in multiple pathways appear to participate in specific elements of the disease, and epistatic interactions among these genes play an important role in both aggravating and suppressing disease development.

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder, classically depicted as a systemic autoimmune disease caused by the production of pathogenic autoantibodies to a spectrum of nuclear antigens. SLE in humans manifests with a diverse array of clinical symptoms that potentially involve multiple organ systems. This heterogeneity reflects direct autoantibody-mediated tissue injury as well as blood vessel inflammation (termed vasculitis) caused by the deposition of complement-fixing immune complexes. A typical patient with SLE is a young woman in her child-bearing years who presents with intermittent fatigue, joint pain and swelling, skin rashes, low white blood cell count, and chest pains due to pleuritis (see Table 1). Approximately one-half of lupus patients will manifest the more severe complications of the disease, which can include nephritis, central nervous system vasculitis, pulmonary hypertension, interstitial lung disease, and stroke. In addition to systemic lupus, there are types of lupus that involve only the skin, as well as a subtype of the systemic disease caused by certain medications (drug-induced lupus).

The diagnosis of SLE is complicated by these extensive variations in clinical symptoms. Current diagnostic guidelines require that patients fulfill any four of 11 criteria to be diagnosed with the systemic form of SLE (Hochberg, 1997b; Tan et al., 1982) (see Table 1). Al-

though this definition creates a phenotypically heterogeneous classification of SLE, nearly all patients with bona fide SLE have elevated titers of antibodies to nuclear autoantigens in serum. As a result, antinuclear antibody (ANA) testing is very sensitive for diagnosing the disease, although not highly specific since ANA antibodies are sporadically detected in as much as 2% of the female population over the age of 40. Antibodies to double-stranded DNA and the Sm protein, on the other hand, are observed essentially only in SLE and, when present, are useful in establishing the diagnosis. In addition, high erythrocyte sedimentation rates, elevated serum levels of acute phase reactants, and low levels of C3 and C4 complement components often accompany active disease. Current treatments for SLE include the anti-malarial hydroxychloroquine, steroids, and cytotoxic drugs such as methotrexate and cyclophosphamide. Although these therapies allow management of disease severity for many patients, a variety of deleterious side effects associated with these drugs together with therapy-resistant disease symptoms significantly diminish the quality of life for many SLE patients.

The worldwide incidence of SLE is conservatively estimated as between 12 and 64 cases per 100,000 individuals, with a striking 9:1 female gender bias and at least 2- to 4-fold higher incidence in non-Caucasian as compared with Caucasian populations (Hochberg, 1997a). There are more than 364,000 women in the US diagnosed with SLE, and possibly an equal number who fulfill two or three of the 11 criteria, but are not diagnosed with SLE. Further, the incidence of disease appears to be increasing. Although the factors responsible for the initiation of SLE are poorly understood, genetic predisposition is firmly established as a key element in susceptibility. Strong familial aggregation in SLE is documented by λ_s estimates for the disease that are well in excess of 15, indicating that siblings of SLE patients have a much greater relative risk for disease in comparison to the population as a whole (Hochberg et al., 1985; Lawrence et al., 1987). In addition, the multigenic nature of SLE susceptibility is indicated by the 10-fold higher rate of concordance for SLE in monozygotic twin pairs (34%) compared with dizygotic pairs (3%) (Block et al., 1975; Deapen et al., 1992).

The genetic basis for susceptibility to SLE has been the subject of intense investigation during the last decade and, although the complexity of systemic autoimmunity remains daunting, several new insights have been obtained both via the analysis of animal models and through linkage analysis in human patient populations. In addition, recent advances in our understanding of the human and mouse genomes are providing tools that will significantly enhance future analyses of complex traits such as SLE susceptibility. Here, we will briefly overview our current understanding of the genetics of both human and murine lupus and discuss strategies and technologies that will impact the future of genetic analysis of SLE.

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Table 1. Criteria for the Diagnosis of Human SLE

Category	Symptom
Skin criteria	Butterfly rash (over the cheeks and nose)
	Discoid rash (scarring rash in sun-exposed areas)
	Photosensitivity
	Oral ulcerations
Systemic criteria	Arthritis (nondeforming)
	Pleuritis or pericarditis
	Proteinuria or cellular urinary casts
	Seizures or psychosis with no other etiology
Laboratory criteria	Hemolytic anemia, leukopenia, lymphopenia, or thrombocytopenia
	Anti-DNA antibodies, anti-Sm antibodies, VDRL false + syphilis test, lupus anticoagulant, or antiphospholipid antibodies
	Antinuclear antibodies (ANA)

Any four of the eleven criteria establish the diagnosis of SLE; revised 1997 American College of Rheumatology (Hochberg, 1997).

Candidate Genes in Human SLE

Given the evidence for a strong genetic influence in SLE, there are hundreds of published studies that have attempted to identify candidate genes contributing to the SLE phenotype (reviewed in Gaffney et al., 2001; Harley et al., 1998; Schur, 1995). The majority of these studies have used classic case/control association methodologies. Although association studies are powerful, they are subject to the problems of population admixture and genetic drift, where differences in allele frequencies between patient populations and control subjects may reflect differences in population history rather than true disease association. Because of admixture and small sample sizes, many of the genes implicated over the years in SLE have not been confirmed and remain controversial. However, the available evidence supports a role for the HLA region, complement components, and low affinity receptors for IgG in the predisposition to human SLE.

HLA

The human leukocyte antigen (HLA) region, spanning 3.6 million base pairs of DNA at 6p21.3, is a gene-rich and transcriptionally active segment that encodes scores of immunologically important genes, including the highly polymorphic MHC class I and class II genes (Caron et al., 2001; Dawkins et al., 1999). Not surprisingly, HLA genes have received significant attention in human SLE, and there is evidence supporting a role for specific extended HLA haplotypes spanning the MHC class II region as genetic risk factors for disease expression in several populations. The DR-B1 alleles DR2 and DR3 have shown consistent associations with SLE in European-Caucasian populations, with a 2- to 3-fold increase in the frequency of these two alleles compared with controls. HLA associations in many non-Caucasian populations, both in the United States and around the world, have not been very convincing or reproducible. Interestingly, DR/DQ alleles show stronger association with the autoantibody profiles observed in SLE than with disease expression itself or individual clinical manifestations (Schur, 1995).

A significant challenge in interpreting these HLA asso-

ciation data, and ultimately in localizing the genetic effect, is posed by the significant linkage disequilibrium (LD) across the region. LD refers to the frequency with which specific sets or "haplotypes" of alleles persist in a population. The reported associations with SLE of several MHC class III genes, including TNF α , the TAP genes, and HSP70, may reflect LD with other genes in the HLA or the epistatic effects of several genes on extended haplotypes.

Fc γ Receptors

The Fc γ receptors Fc γ RI (CD64), Fc γ RII (CD32), and Fc γ RIII (CD16) function to bind and clear IgG antibodies and IgG-containing immune complexes from the circulation. Recent studies have shown that a greater proportion of African-American SLE patients are heterozygous or homozygous for the IgG2 low binding Fc γ RIIIa-R131 allele, with further enrichment observed in patients with lupus nephritis (Salmon et al., 1996). A low binding allele of the Fc γ RIIIa, which functions to bind and clear IgG1- or IgG3-bearing immune complexes, is also associated with SLE and SLE nephritis (Wu et al., 1997). The Fc receptors are very tightly clustered at 1q23, and the interpretation of these results is potentially complicated by LD in the region. Taken together, however, the available evidence is consistent with one or more of the Fc receptor genes contributing to human SLE.

Complement

The complement system consists of approximately 20 plasma proteins that function to mediate inflammatory responses to immune complexes and to assist in the clearance of various infectious microbes (Schur, 1995). For years, a strong relationship has been noted for deficiencies of early classical pathway complement components (C1q, C2, and C4) and the development of SLE (reviewed in Carroll, 1998b; Schur, 1995). Homozygous C1q deficiency is a very rare disorder, and patients suffer from a particularly severe form of SLE with glomerulonephritis and skin manifestations, beginning in the first or second decade of life (Bowness et al., 1994). Deficiencies of C2 and C4 also predispose to SLE (reviewed in Schur, 1995). Both these complement proteins are encoded by genes in the HLA class III region. Complete C2 or C4 deficiencies are rare (one in 10,000 for C2, less than one in 10,000 for C4) and are often associated with a mild form of lupus limited to skin and joint involvement (Arnett and Reveille, 1992). Documenting deficiencies in the C4 genes is complicated by the fact that there are two isotypes of C4—C4A and C4B. Each C4 isotype has numerous allelic variants, and null alleles for each have also been identified. A C4A null allele is transmitted as part of the extended HLA-A7, B8, DR3 haplotype, which, as noted above, is associated with SLE in Caucasian populations (Schur, 1995; Schur et al., 1990). Association studies in African-American and Asian populations, where the null alleles are present on different class II haplotypes, indicate that these C4 alleles are independent risk factors for SLE (Howard et al., 1986; Yamada et al., 1990).

Genetic Linkage Studies in Human SLE

In the early 1990s, several groups initiated efforts to identify the primary susceptibility genes in human SLE

Table 2. Key Characteristics of the Published Human SLE Genome Screens

Study	Number of Families, Individuals, SLE Patients	Family Ethnicity	Primary Method of Analysis	Avg. Intermarker Distance	Reported Risk Loci (total #)	Shared Risk Loci ¹ (total #)	Unique Risk Loci ² (total #)
Minnesota (MN) ³	187 Families 656 Individuals 399 SLE Pts.	148 Caucasian 17 African-Am. 13 Hispanic 9 Other	Nonparametric multipoint linkage	8.9 cM	18	10	8
Oklahoma (OK) ⁴	126 Families 744 Individuals 295 SLE Pts.	77 Caucasian 40 African-Am. 9 Other	Model-based two point linkage/Regression analyses	10 cM	16	7	9
Southern Cal. (USC) ⁵	80 Families 434 Individuals 188 SLE Pts.	43 Hispanic 37 Caucasian	Nonparametric multipoint linkage	12 cM	11	7	4
Sweden (SW) ⁶	17 Families 201 Individuals 44 SLE Pts.	11 Swedish 6 Icelandic	Model-based two point linkage	10 cM	13	3	10

¹ A shared risk locus is a susceptibility locus that is within 10 cM of another reported SLE locus.

² A unique risk locus is a susceptibility locus with no other reported SLE locus within 10 cM.

³ (Gaffney et al., 1998; Gaffney et al., 2000).

⁴ (Moser et al., 1998; Gray-McGuire et al., 2000).

⁵ (Shai et al., 1999).

⁶ (Lindqvist et al., 2000).

families using emerging gene mapping methods. A major hurdle was to recruit sufficient numbers of genetically informative families enriched for systemic lupus. This was challenging given the relatively low frequency of the disease in the population (~1:2,000) and the difficulties in establishing a definitive diagnosis of SLE (see above). Rheumatologists provide medical care for nearly all SLE patients, and they have been extremely generous with their time in assisting the various recruitment efforts. Hundreds of families with two or more SLE cases have now been identified and collected.

To date, there are six published genome-wide screens in SLE families, performed at four different sites. Some of the key features of these studies are shown in Table 2. The 187 University of Minnesota (MN) pedigrees are largely Caucasian (80%) and are exclusively sib-pair families (affected sibs plus all available parents) (Gaffney et al., 1998, 2000). The 126 families of the Oklahoma Medical Research Foundation (OK) collection contain a significant percentage of African-American pedigrees (32%) and about an equal number of sib-pair and extended pedigrees (Moser et al., 1998; Gray-McGuire et al., 2000). The University of Southern California (USC) collection consists of 80 sib-pair families, over half of which are of Mexican-American ancestry (Shai et al., 1999). The University of Uppsala collection in Sweden (SW) consists of 17 large, extended families collected in Iceland and Sweden (Lindqvist et al., 2000). In addition to these screens, a group at UCLA has performed a targeted study examining the evidence for linkage in the 1q41 region, using a collection of 124 multiplex and simplex families of mixed ethnicity (Tsao et al., 1997, 1999). Altogether, these screens have identified 48 distinct chromosomal loci that show at least nominal evidence for linkage in human SLE. Of these, six regions meet criteria for "significant" linkage (LOD score > 3.3), as defined by Lander and Kruglyak (1995) (see Table 3).

Two of the significant regions reside on chromosome 1. The best evidence for linkage at 1q23 is the Fc γ R11a

gene in the OK collection. Similar to the results from the Fc γ R11a association studies, this effect is enriched in African-American families. Supporting evidence for linkage in the region was provided by the USC group. The interval at 1q41-42 shows evidence for linkage in nearly all of the SLE mapping studies reported to date. This locus was originally identified by Tsao et al. (1997), and the effect here was localized close to the poly-ADP ribosyl transferase (PARP) gene, based on strong transmission disequilibrium test (TDT) results with a polymorphic marker in the 5' region of the gene (Tsao et al., 1999). Recent fine mapping in 210 sib pair and 122 trio families from the MN collection has localized the gene in this region just centromeric to PARP near the D1S490 marker (Graham et al., 2001). This region has also been of interest due to synteny of this region with the mouse *Sle1d* locus. In humans, it appears that there are two distinct genetic effects in the 1q41-2 region—one centromeric to PARP at 1q41, and the other closer to the end of the chromosome at 1q42.

The locus at 2q37 showed very strong evidence for linkage in the Swedish and Icelandic families with a LOD of 4.24. Weaker signals were observed nearby in the OK and MN studies, but this could be a locus of particular importance to Scandinavian families. The 4p15 region (marker D4S2366) showed strong evidence for linkage in the OK family collection and was most strikingly demonstrated when a new logistic regression algorithm was applied to the OK dataset (Gray-McGuire et al., 2000). This same marker was subsequently typed in the MN collection and provided supportive evidence for linkage (LOD = 1.50) (Gray-McGuire et al., 2000). There are a number of interesting candidate genes in the region, including CD38, BST1, and ZNF36.

The locus on chromosome 6p21 includes the HLA region, with the strongest evidence for linkage observed in the MN collection and supporting evidence in the USC and OK datasets. As noted above, the HLA was previously implicated in SLE by association studies, and

Table 3. Regions Demonstrating "Significant"¹ Linkage in Human SLE²

Locus	Primary LOD Score	Supporting LOD Score(s)	Candidate Genes	Overlapping Autoimmune Interval	
				Murine Model	Other Autoimmune Disease(s)
1q22-24	OK 3.45 (FcγR)	USC 1.51 ³ (D1S484)	FcγRIIIa	<i>Sle1a</i> & <i>Sle1b</i> (NZM2410)	
1q41-42	OK 3.50 (D1S3462)	USC2.40 ³ (D1S2785) MN 1.92 (D1S235)	PARP	<i>Sle1d</i> (NZM2410) <i>Bxs3</i> (BXSb)	Type I diabetes, Multiple Sclerosis, Rheumatoid Arthritis
2q37	SW 4.24 (D2S125)	OK 1.53 (D2S1363)	INPP5D	<i>Bxs1</i> (BXSb)	
4p15-16	OK 3.84 (D4S2366)	MN 1.50 (D4S2366)	CD38, BST1, ZNF36	<i>Sle6</i> (NZM2410)	
6p11-22	MN 4.19 (D6S426)	OK 1.70 (D6S2439) SW 1.54 (D6S273)	HLA Class II genes, C4a, TNF	<i>Sles1</i> (NZM2410) <i>Lbw1</i> (NZB/NZW)	Multiple autoimmune diseases
16q12-13	MN 3.85 (D16S415)	USC 1.00 ³ (D16S3136)	NOD2		Crohn's Disease, Psoriasis, Type I diabetes

¹ Recommended criteria for significant linkage in a genome-wide scan for a complex trait (LOD ≥ 3.3 for complex pedigrees, LOD ≥ 3.6 for sib-pairs) (Lander and Kruglyak, 1995).

² Shown are LOD scores (marker) meeting criteria for each interval. Supporting evidence (LOD ≥ 1.0) from an independent family collection is also shown if present.

³ Z scores were converted to LOD scores by the equation: LOD = Z²/2ln10.

genes from this region appear to be important in the genetic susceptibility to many, if not all, autoimmune diseases. Recent fine mapping across the HLA using a dense microsatellite screen in the MN family collection has confirmed that the genetic effect is within the HLA itself, and dominant haplotypes have been identified that show strong evidence for both linkage and association.

Finally, the 16q13 region provided a very strong linkage signal in the MN families, a finding supported by data from the USC screen. Importantly, a susceptibility gene for Crohn's disease, an autoimmune inflammatory disorder of the gut, was recently identified in the 16q13 region (Hugot et al., 2001; Ogura et al., 2001). NOD2 is an LPS-responsive gene that influences NF-κB signaling, and a frameshift polymorphism within the coding region of the gene is strongly associated with susceptibility to Crohn's disease. The best evidence for linkage in SLE at 16q13 is precisely at this locus, and NOD2 is currently being examined in SLE families. The 16q13 region has also provided evidence for linkage in psoriasis, type I diabetes, and asthma, consistent with the possibility that certain genes or gene clusters may predispose to different autoimmune diseases in humans (Becker et al., 1998).

In addition to these regions, which meet criteria for "significant" linkage, there are a large number of additional loci that show weaker, but suggestive, evidence for linkage in SLE. The following regions have at least nominal evidence for linkage in at least two independent family collections: 1q31-32 (OK, SW), 2q32 (OK, MN), 3p21 (OK, USC), 6q26-27 (OK, SW), 9q13.3 (OK, SW), 11q23 (OK, SW), 13q31-32 (OK, MN), 18q21 (USC, SW), 20p12-13 (OK, MN), 20q11-13 (OK, MN), and 21q21 (OK, USC).

In summary, the first wave of genomic screens in

human SLE clearly indicates that multiple genes contribute to disease susceptibility in human and that susceptibility is inherited in a fashion similar to other complex genetic diseases. However, some key features of SLE genetics suggest that disease gene identification may be more feasible in this system than in other autoimmune diseases. Although a relatively small number of families have been screened thus far, several loci have been detected that meet criteria for significant linkage, suggesting that at least some of the genes mediating SLE susceptibility in humans are quite potent. In addition, the contribution of HLA to disease susceptibility, although significant, is of a similar magnitude to other susceptibility loci for SLE. Thus, the contribution of HLA to disease will not overwhelm the impact of other loci in linkage analysis. Finally, several regions associated with susceptibility in human studies are syntenic with genomic segments identified by linkage analysis in mice (see Table 3). Although it is not known whether the same genes are mediating disease in both species, the combined fine mapping analysis in both species should cross-fertilize the process of candidate gene identification.

Spontaneous Systemic Autoimmunity in the Mouse
Murine systemic autoimmunity has been recognized as a model of human SLE for over 30 years, and the disease has been extensively characterized in several inbred strains (for review, see Theofilopoulos, 1995a, 1995b). Although specific features of the disease vary among lupus-prone strains, high-titered IgG autoantibodies against a variety of nuclear autoantigens are consistently observed as an element in disease pathogenesis. In addition, hypergammaglobulinemia, splenomegaly, and expanded populations of activated CD4 T cells and B cells are common disease features. Humoral autoimmunity to nuclear antigens is often detectable within the

first 4 to 6 months of age, and disease severity and incidence increases with age. Immune complex-mediated glomerulonephritis culminating in death due to kidney failure is the most severe pathologic complication observed among lupus-prone mice.

Although many parameters of systemic autoimmunity in mice are similar to SLE in humans, there are also several differences. Severe pathogenesis is associated with the production of autoantibodies to double-stranded DNA (dsDNA) in both species; however, autoantibodies against many other nuclear antigens that are strongly associated with disease in humans, such as Sm, Ro, and La, are not associated with lupus nephritis in mice. Also, the severe skin rashes that are often a defining characteristic in human SLE are not observed in murine models, undoubtedly due to dramatic species variations in the anatomy of the epidermis. Finally, the available mouse models do not develop significant levels of many of the severe complications involving the brain and lung that are associated with human disease. This may be a consequence of the relatively short duration of the disease in mice (~6 months) relative to SLE in humans (10+ years). The extended disease course of SLE in humans may mediate a steady deterioration of normal immune regulation in the immune system that ultimately potentiates the development of additional complications. Thus, murine models of systemic autoimmunity are probably most useful for the analysis of genetic mechanisms that initiate the disease process and may not accurately reflect many of the complications that develop in human SLE over extended periods of disease.

Genetic Dissection of Systemic Autoimmunity

Three spontaneous lupus-prone models have been genetically characterized in detail in the mouse. These are: (1) the (NZB X NZW)F1 hybrid (NZB/W) and the related NZM2410 congenic recombinant strain, which is a classic model of spontaneous systemic autoimmunity; (2) the MRL/*lpr* strain, which carries the *lpr* spontaneous mutation of the FAS receptor on an autoimmune-prone MRL background; and (3) the BXSB strain, which carries the Y chromosome autoimmune accelerator (*yaa*) gene on the autoimmune-prone BXSB background. Each lupus-prone strain has strengths and weaknesses as a model of autoimmunity and all vary with respect to their relevance to genetic mechanisms impacting human SLE. The NZB/W model is generally considered to most closely reflect the properties of human SLE, in that this strain exhibits a strong female gender bias in susceptibility and develops a severe systemic autoimmunity culminating in immune complex-mediated fatal glomerulonephritis. The MRL/*lpr* strain also develops systemic autoimmunity and glomerulonephritis, however, the *lpr* mutation, which inactivates FAS-mediated apoptosis and causes a profound lymphadenopathy, is a key element in the penetrance of disease pathogenesis. Although the FAS pathway is an important regulatory pathway in the immune system, mutations in FAS are not associated with susceptibility to human SLE. Susceptibility to lupus in BXSB mice is limited to males because the presence of a Y chromosome carrying *yaa*, which interacts with several genes in the BXSB genome, is essential for the initiation of severe systemic autoimmu-

nity. The *yaa* gene, which has not yet been identified, is a novel and extremely intriguing gene capable of mediating very severe autoimmune phenotypes when incorporated into a permissive genome.

The basis for disease susceptibility in all three of these strains has been analyzed in multiple experimental crosses. Figure 1 summarizes the positions of all the susceptibility alleles that have been detected via linkage analysis in both spontaneous and induced models of SLE (Drake et al., 1994, 1995; Morel et al., 1994, 1999a; Kono et al., 1994; Vidal et al., 1998; Vyse et al., 1996b; Haywood et al., 2000; Hogarth et al., 1998; Rozzo et al., 1996, 2000). Thus far, more than 30 chromosomal regions containing genes impacting lupus susceptibility or resistance have been identified. For every strain analyzed, three or more intervals are associated with disease susceptibility, indicating that murine lupus is genetically complex and mediated by combinations of genes. Interestingly, several of the susceptibility loci mapped to similar locations across multiple strains, most notable in specific regions of chromosomes 1, 4, 7, and the MHC on chromosome 17. Although these results are intriguing and suggest that at least some susceptibility genes may be shared between lupus-prone strains, the majority of the intervals detected in these crosses are strain specific. This indicates that each lupus-prone strain is susceptible in part due to a unique set of disease genes, consistent with the presence of extensive genetic heterogeneity in susceptibility to murine lupus.

A major strength of experimental models is the ability to utilize detailed phenotypic analysis together with sophisticated genetic crosses to analyze the locations and component phenotypes of individual genes. Careful analysis of individual elements in disease pathogenesis, such as the expression of autoantibodies to specific nuclear antigens and the severity of kidney glomerulonephritis, has allowed some susceptibility alleles to be associated with the expression of specific components of disease pathogenesis. Thus, genes such as *Sle1* and *Nba2* in the telomeric region of chromosome 1 are strongly associated with the production of autoantibodies to nuclear antigens (Vyse et al., 1996b, 1997; Morel et al., 1999a). Similarly, *Nba1* on chromosome 4 is not associated with autoantibody production but instead is best correlated with the severity of glomerulonephritis (Vyse et al., 1996a). Several other loci have been associated with mortality and lymphadenopathy (Vidal et al., 1998; Kono et al., 1994). These types of analyses have provided some insights into the roles that individual genes may play in disease pathogenesis and indicate that systemic autoimmunity is mediated by the interactions of several genes with distinct component phenotypes.

The most successful strategy to analyze the contribution of individual susceptibility alleles to a multigenic trait such as systemic autoimmunity has been congenic dissection. This strategy, originally developed by George Snell for the analysis of histocompatibility (Snell, 1948), converts the polygenic system responsible for disease susceptibility into a series of monogenic systems via the production of individual congenic strains, each carrying a single susceptibility interval on the resistant genetic background. The component phenotypes medi-

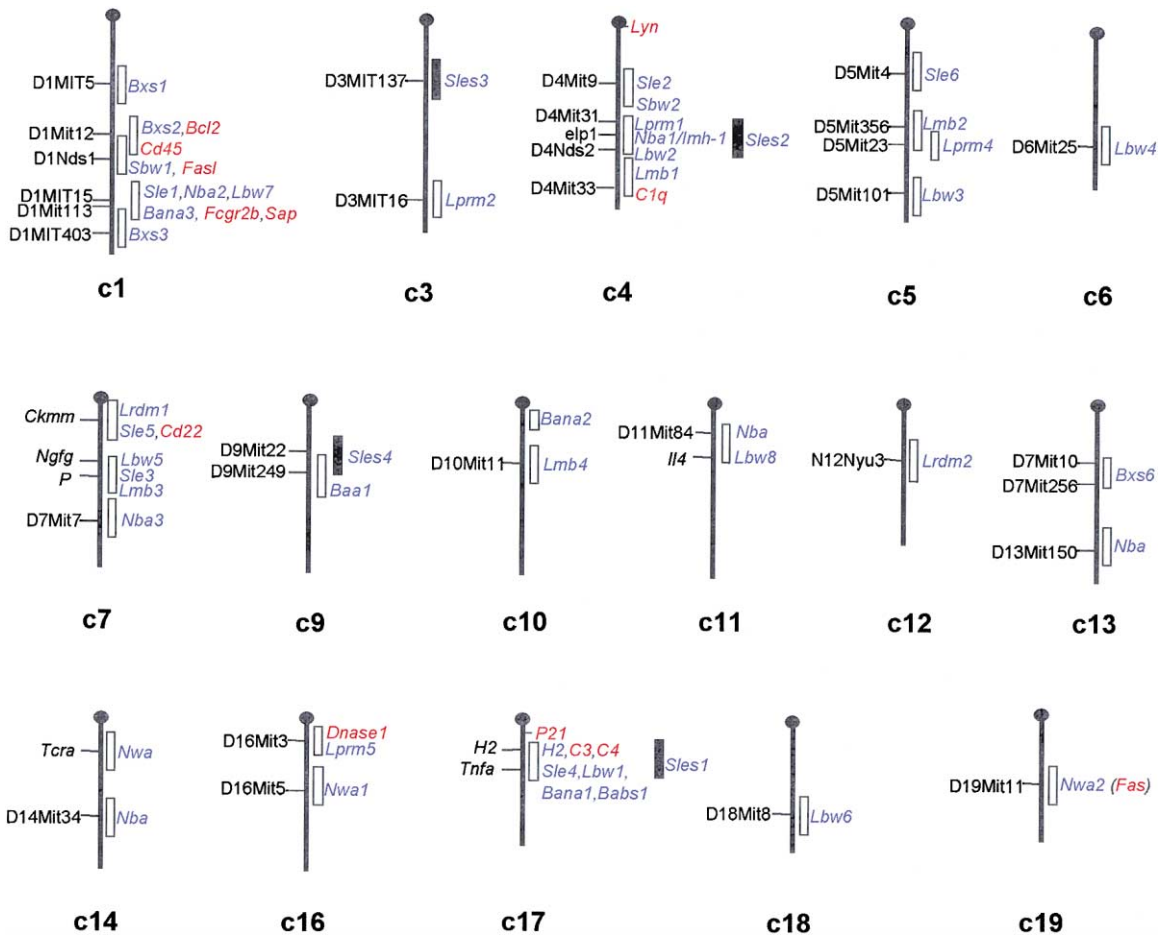


Figure 1. A Diagrammatic Representation of the Mouse Genome Illustrating the Locations of Lupus Susceptibility Genes

Lupus susceptibility loci (blue) are presented with their peak microsatellite markers and support intervals. Loci are from: Drake et al., 1994, 1995; Morel et al., 1994, 1999a; Kono et al., 1994; Vidal et al., 1998; Vyse et al., 1996b; Haywood et al., 2000; Hogarth et al., 1998; Rozzo et al., 1996, 2000. The positions of suppressive modifiers are presented with black bars to define their support intervals (Morel et al., 1999a). The positions of genes that have been reported to mediate systemic autoimmunity when disrupted are shown in red and their linkage positions are from the Mouse Genome Database.

ated by each individual susceptibility gene can then be analyzed separately via the phenotypic analysis of individual congenic strains. In addition, component phenotypes detected in individual congenic strains are amenable to genetic and functional analysis as Mendelian traits, thus making traditional fine mapping and subsequent positional cloning strategies feasible.

The most extensive use of congenic dissection to investigate the properties of individual susceptibility alleles in lupus-prone strains has been with the NZM2410 strain (Morel et al., 1997; Wakeland et al., 1997). Susceptibility to lupus in NZM2410 is predominantly due to genes localized to the telomeric region of chromosome 1 (*Sle1*), the middle of chromosome 4 (*Sle2*), and the centromeric segment of chromosome 7 (*Sle3*) (Morel et al., 1994). Congenic strain construction was performed by transferring each of these intervals from NZM2410 onto the B6 background, and phenotypic analysis revealed that each locus contributes a unique component phenotype to disease pathogenesis (Morel et al., 1997). *Sle1* mediates the loss of tolerance to nuclear antigens with a high specificity for the H2A/H2B/DNA subnucleo-

some (Mohan et al., 1998). *Sle2* lowers the activation threshold of B cells, leading to polyclonal IgM antibodies (Abs) and expansion of the B1a cell compartment (Mohan et al., 1997). *Sle3* mediates a T cell dysregulation that is associated with polyclonal IgG Abs and a decrease in activation-induced cell death in CD4⁺ T cells (Mohan et al., 1999b).

Although the B6.*Sle* congenic strains express component phenotypes relevant to autoimmunity, none develop severe pathology, indicating that the individual genes are not sufficient to cause lupus. To assess the fashion in which these genes interact to mediate more severe autoimmune phenotypes, the individual congenic intervals were reassembled in various combinations on the B6 background (Mohan et al., 1999a; Morel et al., 2000). Interestingly, the coexpression of *Sle1*, *Sle2*, and *Sle3* as a B6-triple congenic results in severe systemic autoimmunity and fully penetrant, fatal glomerulonephritis (Morel et al., 2000). These results demonstrate the fulfillment of the genetic equivalent of Koch's postulate, where susceptibility loci in a lupus-prone strain have been identified by a genome scan, isolated and

functionally characterized by congenic dissection, and finally shown to mediate full disease expression when recombined in a normal genome. This analysis additionally demonstrated that *Sle1*, which breaks tolerance to chromatin antigens, was essential for the development of fatal lupus nephritis in the B6-congenic model system since only combinations of intervals that included *Sle1* developed severe lupus nephritis.

A fine mapping analysis of the *Sle1* congenic interval was recently completed, and this analysis has revealed that a single susceptibility interval can be highly complex (Morel et al., 1999a, 2000). Phenotypic analysis of over 250 recombinants spaced throughout the *Sle1* congenic interval detected four separate susceptibility loci; three capable of breaching humoral immune tolerance to chromatin antigens and one mediating severe glomerulonephritis when combined with other susceptibility alleles in a permissive genome (Morel et al., 2000). Thus, the “*Sle1*” phenotype actually represents the combined effects of four separate loci, each contributing a subset of the component phenotypes detected in B6.*Sle1*. The detection of a cluster of genes mediating a complex trait phenotype, rather than a single gene, has been a consistent observation of fine mapping studies on individual loci contributing to autoimmune disease (for review, see Wandstrat and Wakeland, 2001). As listed in Table 3, *Sle1a* and *Sle1b* localize into regions syntenic with 1q21-23, and *Sle1d* into a region syntenic with 1q41. Thus, the *Sle1* cluster appears to contain genes that are syntenic with two of the six human intervals that exhibit significant linkage with susceptibility to SLE. These results raise the possibility that similar genes or possibly a gene family are responsible for susceptibility to systemic autoimmunity in both mice and humans, although further work will be necessary to delineate the relationship of the *Sle1* gene cluster with SLE susceptibility genes in human 1q23 and 1q41.

Epistatic Interactions and Systemic Autoimmunity

Epistasis is classically defined as a genetic interaction in which the genotype at one locus affects the phenotypic expression of the genotype at another locus (Suzuki et al., 1989). The importance of epistasis in the development of severe systemic autoimmunity was apparent early in the analysis of lupus-prone mouse models, in that (NZB X NZW)F1 hybrid mice were found to develop severe systemic autoimmunity, although both parental strains were not severely autoimmune. Thus, genetic interactions between alleles in NZB and NZW resulted in the expression of a phenotype (severe systemic autoimmunity), which was absent in both parental strains. Similarly, the *lpr* spontaneous mutation of Fas, which is a potent autoimmune susceptibility gene in the MRL/*lpr* lupus model, lost its autoimmune phenotype when introgressed onto other genetic backgrounds (Fossati et al., 1993; Warren et al., 1984). Similar findings have been made with the *yaa* gene in BXSB. These relationships illustrate that the autoimmune potential of specific susceptibility genes is exquisitely dependent upon the presence of a “permissive” genome to potentiate the expression of an autoimmune phenotype.

The spontaneous autoimmune phenotypes of the congenic B6.*Sle1*, B6.*yaa*, and bicongenic B6.*Sle1/yaa*

strains provide a clear illustration of synergism between susceptibility alleles for lupus. B6.*Sle1* and B6.*yaa* spontaneously produce nonpathogenic autoantibodies to nuclear antigens, but fail to develop severe autoimmunity. However, when these two susceptibility alleles are combined in the B6.*Sle1/yaa* bicongenic strain, a severe systemic autoimmunity develops, culminating in fatal glomerulonephritis with an incidence of 70% by 9 months of age (Morel et al., 2000). This illustrates an epistatic interaction between two susceptibility alleles that causes a greater increase in disease severity than would be predicted by simply adding their individual phenotypes together.

A second type of epistasis, in which epistatic modifiers suppress the autoimmune phenotypes of susceptibility alleles, has also been detected using the B6.*Sle* congenic strains (Morel et al., 1999b). Genes capable of suppressing autoimmunity were detected via the analysis of the disease phenotype mediated by *Sle1*, *Sle2*, and *Sle3* when introgressed onto different genetic backgrounds. As discussed above, B6.*Sle1/Sle2/Sle3* triple congenic mice develop fatal lupus nephritis with a penetrance approaching 100% in both genders by 9 months of age (Morel et al., 2000). Interestingly, all three of these susceptibility alleles are derived from the NZW genome, and yet, as discussed above, NZW exhibits only very benign autoimmune phenotypes in females greater than 12 months of age (Kelley and Winkelstein, 1980). Thus, the phenotypic expression of *Sle1/Sle2/Sle3* is significantly suppressed in NZW. A linkage analysis found four separate loci that accounted for the suppression of lupus susceptibility in NZW (Morel et al., 1999b). These results indicate that the disease mediated by susceptibility genes can be fully suppressed by other “modifying” genes in the genome. Interestingly, *Sles1*, which is the most potent suppressive modifier detected in this analysis, was shown to specifically suppress the autoimmune phenotype of *Sle1*. This result is especially intriguing because *Sles1* is located in close proximity to the murine MHC, which places it in an area that is syntenic with another potent SLE susceptibility interval detected by human linkage analysis of SLE (Table 3). Although the extent of epistasis in human SLE remains to be determined, it is reasonable to predict that epistatic interactions are a consequence of the many functional polymorphisms impacting immune recognition and responsiveness and will therefore be a component of SLE susceptibility in most species.

Genetic Pathways Implicated by Synthetic Models of Autoimmunity

A collection of synthetic models of autoimmunity have been created via targeted genetic disruptions of specific regulatory genes in the immune system and/or the creation of transgenic strains with aberrant/overexpression of regulatory pathways. The strength of this approach is that it allows an *in vivo* assessment of the impact of severe modifications in the expression of these genes on the immune system. In addition, these synthetic models can be used in some instances to characterize an effector mechanism involved in disease pathogenesis and/or to delineate genetic mechanisms that will lead to systemic autoimmunity.

Table 4. Candidate Genes and Pathways Implicated in SLE

Proposed Mechanism	Murine SLE	Human SLE
Antigen/Immune complex clearance	C1q knockout	C1q
	C3, C4 knockout	C2, C3, C4
	Sap ¹ knockout	Mannose binding protein
	DNase I knockout	Fc γ RIIA
	Serum IgM knockout	Fc γ RIIIA
	Fc γ common chain knockout	DNase I
	Mer knockout	
Lymphoid signaling	SHP-1 knockout	T cell receptor ζ chain
	Lyn knockout	TNF α
	Lyn/Fyn double knockout	IL-10
	CD22 knockout	
	BlyS transgenic	
	PD-1 knockout	
	IL-2 knockout	
	CD45 E613R point mutation	
	G2A knockout	
	IFN-gamma transgenic	
Apoptosis	Fas knockout	
	Fas-L knockout	
	Bcl-2 transgenic	
	Pten ² heterozygous deficiency	
	p21 cyclin dependent kinase knockout	
Epitope modification	α -Mannosidase II knockout	

¹ Serum Amyloid P-component.

² Phosphatase and tensin homolog.

Table 4 provides a listing of modified genes that have been reported to mediate some level of systemic autoimmunity and their locations in the mouse genome are presented in Figure 1. These genes can be organized into four broadly defined pathways impacting immune functions relevant to systemic autoimmunity. The first pathway affects the clearance of nuclear chromatin or immune complexes. Deficiencies in C1q, C3, and C4 have all been shown to mediate the development of various levels of humoral autoimmunity to nuclear antigens. The targeted disruption of the C1q gene by Walport and coworkers was the first complement component deficiency to be shown to mediate systemic autoimmunity (Botto et al., 1998). Pathologic studies also revealed that large numbers of apoptotic cells accumulated in diseased glomeruli, supporting the hypothesis that C1q may have a critical role to play in the physiological clearance of apoptotic cells. Kelsoe and colleagues recently reported that the targeted disruption of the gene encoding C4 led to the spontaneous development of lupus-like autoimmunity, a result very similar to that for C1q (Chen et al., 2000). These results document the ability of deficiencies in multiple complement components to mediate a breach in humoral tolerance to nuclear antigens (Carroll, 2000; Chen et al., 2000). Although the precise pathway involved is unclear, a role for the CR1/CR2 receptors was excluded via the analysis of a Cr2^{-/-} mutant, which failed to develop autoimmunity (Applequist et al., 2000). Some investigators have postulated that these complement components play a role in the physiology and metabolism of apoptotic cells and/or chromatin and that their deficiency leads to the generation of an autoimmune-prone B cell receptor repertoire during B cell ontogeny (Carroll, 1998a). Although intriguing, this hypothesis lacks formal proof. However, recently other investigators have shown that deficiencies

of either Serum amyloid P component (SAP) (Bickerstaff et al., 1999), a protein involved in the metabolism of chromatin, or DNase I (Napirei et al., 2000), a molecule potentially involved in chromatin metabolism, both lead to modest levels of systemic autoimmunity. Interestingly, polymorphisms in the DNase I gene have recently been detected in SLE patients, suggesting that polymorphisms in this gene may also potentiate SLE in humans (Yasutomo et al., 2001). These findings further support the conjecture that modifications in chromatin metabolism may predispose for the development of humoral autoimmunity to nuclear chromatin (Walport, 2000).

The second pathway potentiating the development of systemic autoimmunity includes cytokines and signal transduction molecules that regulate immune activation. Targeted disruptions of *SHP-1*, *Lyn*, *CD22*, *PD-1*, *IL-2*, *G2A*, the Fc γ common subunit, and *CD45* all have been shown to cause varying levels of humoral autoimmunity to nuclear antigens, especially when bred onto permissive genetic backgrounds (Nishimura et al., 1999; Cornell et al., 1998; Bolland and Ravetch, 2000). Transgenic dysregulation of *BlyS* and IFN γ have a similar effect (Khare et al., 2000). A third pathway impacting systemic autoimmunity involves a collection of genes with various roles in the regulation of apoptosis. As discussed above, disruptions of the FAS pathway are known to dramatically impact autoimmunity in the MRL background. A dysregulated *bcl-2* transgene also was reported to breach tolerance to nuclear antigens, at least when bred onto specific backgrounds (Mandik-Nayak et al., 2000). Finally, targeted disruptions of genes impacting glycosylation have been shown to mediate a strong systemic autoimmunity (Chui et al., 2001). The precise molecular mechanism responsible for this effect remains to be elucidated.

Perhaps the most puzzling aspect of the results with

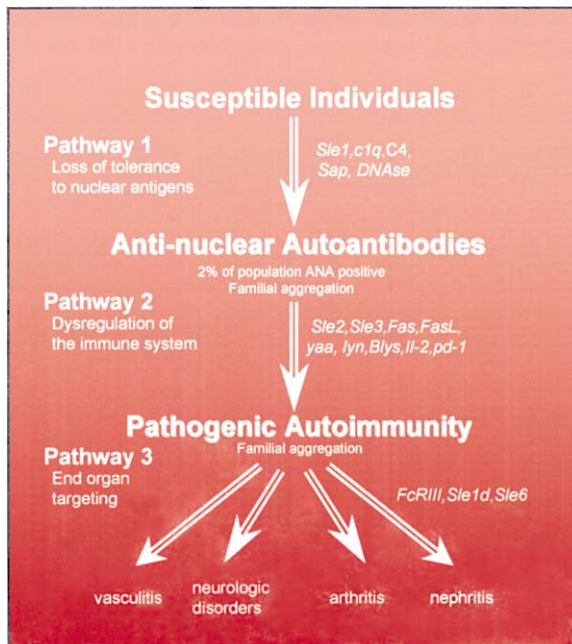


Figure 2. A Hypothetical Pathway Illustrating the Manner in which Individual Susceptibility Genes Interact to Potentiate Severe Autoimmunity

targeted genetic disruptions is the overall frequency with which these manipulations have revealed a predisposition to systemic autoimmunity. In this regard, three separate reports have clearly documented that B6 X 129 hybrid genomes are spontaneously predisposed to the development of humoral autoimmunity with low levels of glomerulonephritis, especially in aged females (Botto et al., 1998; Bickerstaff et al., 1999; Santiago-Raber et al., 2001). As a result, both control and experimental mice would be predicted to express a background level of systemic autoimmunity when targeted disruptions are tested in mice segregating a mixture of the B6 and 129 genomes (a very common situation). In fact, a recent analysis of a p21 disruption reported a much less robust association with systemic autoimmunity than was previously described, predominantly due to the use of appropriate control animals (Santiago-Raber et al., 2001). Given these findings, the impact of epistatic interactions between the B6 and 129 genomes on systemic autoimmunity should be carefully assessed when analyzing the autoimmune phenotypes of specific genetic disruptions.

Genetic Interactions during SLE Pathogenesis

A three-step hypothetical model of the roles that various genetic pathways play in the initiation of SLE pathogenesis is presented in Figure 2. The development of SLE can be viewed as involving interactions between genes in three separate pathways. The first pathway contains genes such as *Sle1*, *C1q*, *C4*, *SAP*, and *DNase* that can trigger the loss of immune tolerance to nuclear autoantigens and mediate the initiation of autoimmunity. In this regard, many first-degree relatives of SLE probands exhibit a similar seropositive phenotype without severe disease pathogenesis. At the second step of the path-

way, genes like *Sle2*, *Sle3*, *Fas*, *FasL*, *yaa*, *lyn*, *Blys*, *Il2*, and *pd-1* participate in the process of hypersensitization and/or disruption of the normal immune system, leading to immune dysregulation. Some genes in this pathway are capable of causing the initiation of a humoral autoimmune response to nuclear antigens that is minimally pathogenic. When added to a gene in the first pathway, however, they will mediate a transition to pathogenic autoimmunity to nuclear antigens (Mohan et al. 1999a). Finally, *FcγIIIa*, *Sle6*, and *Sle1d* are genes that we would propose to be involved in the final step of the pathway. Genes in this step mediate ANA targeting into the specific organ to promote end organ culmination. Theoretically, end organ damage, for example of the kidney, can be mediated by a variety of factors, including molecules or mechanisms that affect or dictate glomerular architecture, the structural and chemical makeup of the basement membrane, local inflammatory processes, local cytokine production, and miscellaneous factors that control immune complex clearance.

Future Prospects for Genetic Analysis of SLE

A crucial goal for future efforts in the genetics of SLE will be to transition from linkage analysis into gene identification and the characterization of genetic mechanisms mediating autoimmunity. The recent publication of an almost complete nucleotide sequence of the human genome has provided detailed physical and molecular maps of the majority of human linkage groups (Venter et al., 2001). In addition, a growing database of single-nucleotide polymorphisms (SNPs) together with an improving technology for their detection will greatly enhance the statistical power of linkage analysis (Halushka et al., 1999; Cargill et al., 1999). These technological advances in molecular genetics should provide the tools needed to begin to delineate the genetic mechanisms responsible for susceptibility to SLE in human populations. However, the complexity of inheritance and genetic heterogeneity of SLE susceptibility remains a daunting challenge, and success in the identification of susceptibility alleles in human populations will probably await the development of more powerful analytical procedures and larger patient populations.

The identification of lupus susceptibility genes in animal models, on the other hand, is clearly imminent. Congenic dissection is a powerful tool that has allowed susceptibility genes to be precisely localized into intervals as small as 800–1000 kb (Lyons et al., 2000; Morel et al., 2001). The soon to be completed mouse genome project will dramatically simplify the identification of positional candidates in these intervals, and definitive gene involvement can be determined through *in vivo* complementation using BAC transgenic technologies (Antoch et al., 1997; Probst et al., 1998). Targeted mutagenesis in BACs and/or ES cells can also help to definitively identify specific positional candidates as susceptibility genes.

Finally, the use of gene expression microarrays to identify genes whose expression is modified by SLE susceptibility alleles has the potential to revolutionize mapping strategies for complex traits. Theoretically, this technology should identify genes that are dysregulated during the initiation of systemic autoimmunity, thus pro-

viding new insights into disease mechanisms and expanding the spectrum of potential targets for the development of therapeutic strategies. This technology may also identify molecular expression phenotypes that allow the identification of individuals who are at risk of developing disease, thus affording them the opportunity for preventive health care measures.

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