

A genetic analysis of lupus

Systemic lupus erythematosus (SLE) is a complex multigenic inherited disease with susceptibility determined by a combination of genetic, environmental and stochastic factors. Although not yet defined, recent technical advances have provided the means to dissect the component genetic contributions of polygenic traits. We have applied such approaches to mouse models of spontaneous SLE and, in this report, summarize our genome wide mapping studies that identified loci predisposing to several major lupus-related traits. Through the generation and study of interval congenic lines, precise mapping, and screening of candidate genes, identification of the specific genes and mechanisms associated with some of the major loci is currently being pursued.

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Key words: autoimmunity, lupus, animal models, genetics.

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Introduction

The familial risk of developing complex disorders, such as lupus, can be expressed by the parameter λ_s (risk of siblings/prevalence in general population), which reflects the combined risk of genetic susceptibility and shared environments. For SLE, the calculated λ_s is in the region of 20, i.e. well above the value of 1.0 that would be expected in the absence of familial clustering (1). Although the relative contributions of environment and genetic factors are not known, family and twin studies have clearly indicated that genetic factors are important. These findings along with unequivocal evidence of genetic influence in animal models of lupus, provide a strong impetus for cloning the predisposing alleles in this disease.

Disease-promoting susceptibility genes can be identified either by their functional characteristics (candidate screening) or, on the other extreme, solely by chromosomal position (positional cloning). The former requires prior knowledge of not only the gene, but also the specific relevant activity. In contrast, identification based on genome location requires no preconceptions about the specific gene or its actions. For this reason this latter approach is especially appealing for investigating idiopathic diseases such as SLE where the nature of susceptibility genes is not known. This, however, entails not only mapping, but eventually cloning of the entire specific chromosomal segment and is effort and technically demanding. Increasingly, however, an intermediate approach is applied, which uses the chromosomal location as the initial step, followed by picking candidate genes that have already been

identified, mapped, and analyzed in their structural and expression characteristics, as part of the genome initiative (positional candidate screening).

Mapping traits

There are two general approaches to map genes in human populations, allele sharing methods (linkage analysis) and association studies. The former uses sibling-pairs or extended pedigrees to search for enrichment of marker genotypes in affected individuals. Loci are identified because of cosegregation (linkage) of closely placed sequences (identity-by-descent). Since only one or a few generations are involved, linkage typically occurs over several centimorgans making it practical for genome-wide scans. Power to detect loci is strengthened with increased penetrance, but reduced by genetic heterogeneity and high allele frequencies. This approach has been highly effective in sorting out Mendelian disorders, but is limited to identifying complex genetic loci with moderate to large effects.

Association studies essentially compare the frequencies of an allelic gene or genetic marker in affected and control populations. Specialized families are not required and large numbers of subjects can be assimilated making it possible to more readily detect genes with small effect. Careful attention must be directed toward matching the age, sex, genetic composition and environmental exposure of the two groups since unforeseen disparities are often the cause of false positive assertions. Since individuals in the two populations are separated by a large number of

generations the linked distances are usually small and in the kilobase range. This is useful for precise mapping of loci, but a substantial drawback for genome-wide screening where simulations suggest well over 100 000 markers may be required (2).

Genome-wide screens are only possible because of the availability of large numbers of highly polymorphic DNA markers. One type of marker in common use today, particularly in linkage analysis studies, are microsatellites. These consist of di-, tri-, or tetranucleotide repeats, most commonly dinucleotide repeats of CA, that range in size from a few to several hundred repeats. There are over 100 000 of these dispersed randomly throughout the genome and at each site they are generally polymorphic in length (number of repeats) among individuals in a population.

Another type of polymorphic marker is single nucleotide polymorphisms or SNPs (2, 3). SNPs are generally common alleles present in the population at a frequency of >1%. SNPs are responsible for most of the heterozygosity in the population, a reflection of the relatively limited diversity of the human population presumably because of its relative youth and small size. Consequently most SNPs because they are present in diverse populations are likely 'ancient' alleles that originated prior to separation of population groups. In contrast, infrequent variants are the main causes of rare, highly penetrant Mendelian genetic diseases and are typically more recent in origin. In part because of their above characteristics, high density in the genome (several million), and relative ease of detection (automatable), as well as the fact that many of the disease-promoting alleles are likely to be caused by point mutations, enthusiasm has shifted from using linkage analysis to define complex genetic disorders to using dense SNP maps in association studies. Such studies can be divided into direct or indirect forms. The former examines whether the specific mutation (the actual SNP) causes disease by testing for increased frequency of the mutation in affected populations. The indirect form uses densely spaced SNPs to scan regions for ancient SNPs in linkage disequilibrium with the specific mutation. Such SNPs will be found in higher frequency in affected individuals. This approach does not require the discovery of every disease causing allele, but requires a very dense SNP map (500 000 or more SNPs), which under current genotyping capacity is not yet feasible for large scale screening (4). Success of this approach also rests on largely untested assumptions about the relationship of SNPs and common disease-inducing mutations in the population. Currently efforts are being directed toward defining the overall landscape of SNPs in human populations, as well as the more limited approach of screening candidate SNPs particularly those that alter the protein sequence (3).

Identification of susceptibility genes in animal models

A complementary approach to defining the genetics of complex disorders is to define it in animal models and to apply these findings to the more complex human situation. This takes advantage of the fact that genetic studies are more powerful in animal models particularly mice because of a number of factors, including, homogeneous genetic backgrounds, standard environmental conditions, accurate phenotyping, the ability to breed the numbers and most informative crosses and the ability to alter the genome by genetic engineering (congenic, transgenic, gene knockout). The ability to manipulate the mouse genome makes it possible to directly test the significance of specific putative susceptibility genes. In fact, generation of mutations found in humans by either modifying the homologous mouse gene or by directly inserting a region of human genome containing the altered gene are approaches to confirm human susceptibility alleles.

Although the relevance of animal mutations for the human disease will not be known until after they have been cloned, it is likely given the much greater genetic heterogeneity in humans that closely related mutations will be found in at least some population. Furthermore, identification of pathways critical for the induction and development of lupus will be of considerable importance in efforts to dissect disease pathogenesis.

Identification of susceptibility genes in animal models of complex disorders can be divided into five general steps. First, the trait is mapped to a chromosomal region using a genome-wide set of evenly spaced markers. This entails selection of a susceptible (S) and a resistant (R) strain, testing F1 (SxR) mice to determine the mode of transmission, and setting up either backcrosses (F1xS or F1xR) or intercrosses (F1xF1). Usually for dominant or recessive transmissions a backcross is best since the number of mice required are less since there are only two possible genotypes (S or R and F1) at each locus. F2 intercrosses, on the other hand, are particularly useful to identify all of the contributing loci and to define the mode of inheritance and epistasis since all combinations of alleles will be present (S, R, F1). Selection of the phenotypes resulting in a large variance (wider range) in the experimental crosses generally increases the power to detect quantitative trait loci (QTL; loci that contribute to only a portion of the total trait). Linkage of traits to markers are assessed using a variety of methods resulting in typically 10–20 cM resolution (≈ 20 –40 million basepairs). In the second step, the complex genetic disease is reduced to simple component Mendelian traits by the generation of independent congenic lines, each containing a single QTL. Generally if a measurable phenotype can be found the underlying susceptibility gene can be cloned. It is important that the component phenotype be

related to the original trait. A caveat, however, is that the effects of closely situated genes within the same locus will usually not be resolvable at this stage. In the third step, the size of the QTL interval is reduced by testing a sufficient number of recombinants (usually in the hundreds). The approaches depend on the strength of the QTL and for those with weak effect, this may require generating sets of congenic sublines with smaller overlapping intervals. The fourth step identifies the specific susceptibility allele. This entails identifying the genes in the reduced interval and screening the most likely candidates for functional alterations consistent with the component phenotype. Cloning of the entire interval is required if there is no known candidate gene. Reducing the QTL to as small an interval as possible and having a detailed characterization of the QTL phenotype in order to select the candidate genes, greatly assist this process. The final step is to prove the identity of the specific allele. This can be done in some instances using transgenic or gene knockout approaches. These procedures are technically demanding especially if they involve nonstandard strains of mice.

Loci predisposing to systemic autoimmunity in lupus-prone mouse strains

Only a few susceptibility genes have been identified in mice with spontaneous SLE. These include the MHC, the Fas (*lpr* and *lpr^{cg}* mutations) and related Fas ligand (*gld* mutation) genes, and the Y chromosome associated with accelerated autoimmunity (*Yaa*) gene in BXSB male mice that has not yet been cloned. In all instances, however, the development of lupus-like disease requires the presence of other still undefined lupus-predisposing background genes that appear to shape the severity, autoantibody profiles and types of end-organ disease. In regard to the MHC, susceptibility is not restricted to a particular haplotype, but is dependent on the strain background (5, 6). The specific gene or genes responsible for the MHC association is still a matter of controversy, although both class II (6, 7) and TNF α (8, 9) genes have been suggested as candidates.

NZ susceptibility loci

Our initial genome-wide linkage study of (NZB $\times$ NZW) F_2 mice revealed 8 loci designated *Lbw1*–8, that were located on chromosomes 17, 4, 5, 6, 7, 18, 1, and 11, respectively, and linked to one or more component phenotypes, including mortality, glomerulonephritis (GN), IgG antichromatin autoantibody production and splenomegaly (Table 1). *Lbw1* was linked to mortality, GN and antichromatin autoantibodies and mapped to the MHC region. Heterozygosity at *Lbw1* (H-2^{d/z}) conferred the greatest susceptibility than either H-2^d or H-2^z haplotypes

consistent with previous studies (10, 11). NZB (H-2^d) and NZW (H-2^z) class II genes (12–16), the NZW TNF- α gene (9, 17), or both (8) have been suggested as candidates for this locus. Recently, a recessively transmitted NZW locus, designated *Sles1*, that also mapped to the MHC region was suggested to act as an epistatic modifier that suppressed autoimmune disease in the NZW strain (18). Whether findings related to *Sles1* are simply due to the MHC region polymorphisms noted in previous studies or to a novel suppressor gene remains to be determined.

Four of the seven remaining *Lbw* loci (*Lbw2*, *Lbw3*, *Lbw5*, and *Lbw7*) have been confirmed by other crosses or by interval-specific congenic mice. *Lbw2* on chromosome 4 is a dominantly transmitted NZB locus linked to mortality, GN and splenomegaly (19). Other groups have also linked this chromosomal interval to these (*Nba1*) (20) (*Sle2*) (21), and other traits, including hyper IgM production (*Imh1*) (22), B cell hyperactivity (23), increased B1 cells (23, 24) and enhanced Mott cell formation from B1 cells (*Mott1*) (25). The NZW *Lbw3* locus on chromosome 5 was linked to mortality in both the BWF $_2$ and (BXSB $\times$ NZW) F_2 crosses (below). *Lbw5*, on chromosome 7, was a dominantly transmitted NZW locus linked to mortality that overlapped with *Sle3* (21), a NZW QTL linked to autoantibodies and GN (26) and a NZW locus predisposing to splenomegaly (BXSB $\times$ NZW) F_2 cross below). *Lbw7* on chromosome 1, overlaps with *Sle1* (21) and *Nba2* (27–29). *Lbw7* and *Nba2* are inherited from the NZB strain and likely represent the same locus, whereas *Sle1* is from the NZW genome and therefore different. Interestingly, both the NZB (*Lbw7* and *Nba2*) and NZW (*Sle1*) loci are linked to antichromatin production, however, the NZB locus is also linked to the production of autoantibodies to the endogenous retroviral glycoprotein, gp70 (29). Crosses between NZB and NZW strains have not detected linkage to the chromosome 1 region of the NZW (*Sle1*), which suggests that the overall contribution of *Lbw7/Nba2* (NZB locus) may be greater than *Sle1* at least in the NZ background.

Inheritance of autoimmune traits in the (NZB $\times$ NZW) F_2 mice appeared dependent on both the number and specific combinations of susceptibility

Table 1 Summary of (NZB $\times$ NZW) F_2 -derived lupus susceptibility loci

Chr	Name	Strain	Traits
1	<i>Lbw7*</i>	NZB	Antichromatin Ab, splenomegaly
4	<i>Lbw2</i>	NZB	Mortality, GN, splenomegaly
5	<i>Lbw3</i>	NZW	Mortality
6	<i>Lbw4</i>	NZB	Mortality
7	<i>Lbw5</i>	NZW	Mortality
11	<i>Lbw8</i>	NZB	Antichromatin Ab
17	<i>Lbw1</i>	NZW/NZB	Antichromatin Ab, mortality, GN
18	<i>Lbw6</i>	NZW	Mortality, GN

*Loci from other studies probably identical to *Lbw* loci: *Lbw7* (*Nba2*) (27–29), *Lbw2* (*Nba1*, *Sle2*, *Imh1*, *Mott1*) (20–22, 25), *Lbw5* (*Sle3*) (21, 26).

alleles (multiplicative epistasis model), which has been supported by subsequent studies (18, 27, 30). It was also noted that some loci predisposing to mortality were not linked to autoantibody production, nor in some cases to nephritis. This suggested that NZ genes may contribute to systemic autoimmunity not only by promoting loss of tolerance and autoantibodies, but also by influencing disease pathogenesis at later stages. The specific mechanisms that could account for this are not known, however, this type of model is supported by three recently described genetically modified lupus-prone mice: the (NZB $\times$ NZW) F_1 hybrid deficient for the FcR γ chain (31, 32) and MRL-*Fas*^{lpr} mice either heterozygous for the IFN- γ knockout (33) or lacking the monocyte chemoattractant protein-1 chemokine (34). In these animals, levels of serum autoantibody and kidney immunoglobulin deposits were similar to wild-type lupus mice, however, there was minimal to no GN, possibly because the knockout mutations blocked an important secondary inflammatory process.

To further define the role of the non-MHC dominant susceptibility loci (*Lbw2*, *Lbw5* and *Lbw7*) in lupus-like disease, reciprocal interval-specific congenic mice are being studied. This strategy allows examination of *Lbw* loci in both parental strain backgrounds and in the (NZB $\times$ NZW) F_1 hybrid, the model considered to most closely resemble human SLE. Moreover, since the component phenotypes ascribed to specific loci are dependent to varying degrees on the genetic background of the host strain, a more comprehensive perspective on the role of susceptibility loci may be accomplished using this approach. Furthermore, the effect of replacing the susceptible chromosomal interval with the nonsusceptible interval on the severity and manifestations of disease would provide an indication of the potential benefit of intervention. Initial studies focused on *Lbw2* on chromosome 4, using congenics with the NZW *Lbw2* interval on the NZB background (NZB.NZW-*Lbw2*) and the reciprocal (NZW.NZW-*Lbw2*) (Haraldsson et al. unpublished data). *Lbw2*'s role in (NZB $\times$ NZW) F_1 mice was determined by analyzing F_1 hybrids with no, one or two copies of the susceptible NZB *Lbw2* interval. In close agreement with the (NZB $\times$ NZW) F_2 mapping data (19), *Lbw2* was dominantly transmitted and associated with accelerated mortality and nephritis, splenomegaly, but not with increases in levels of IgG antinuclear antibodies. Importantly, BWF1 mice lacking the NZB *Lbw2* interval had a significant improvement in one year survival compared with wild type F_1 hybrids increasing from approximately 10% to 50%. From a therapeutic standpoint, this clearly shows that correction of a single non-MHC predisposing locus can have a dramatic effect on overall disease course despite the other remaining genetic contributions to disease. Whether this is due to one or perhaps more predisposing genes within the *Lbw2* interval is currently being assessed.

The interval-specific congenic NZB.NZW-*Lbw2* mice, lacking the NZB *Lbw2* interval, also exhibited many of the above reductions in disease manifestations, but, in addition, had delayed and reduced Coomb's positivity compared to the wild type NZB, which characteristically develops severe, sometimes fatal, Coomb's positive hemolytic anemia. This finding verified the presence of a previously described locus linked to autoimmune hemolytic anemia in NZB mice (*Aia-1*) that was mapped to chromosome 4 distal to *Lbw2* (35). In contrast, the reciprocal NZW.NZW-*Lbw2* congenic, despite expressing the NZB *Lbw2* interval, never developed Coomb's positivity even up to 16 months of age. Thus, *Lbw2* is important but not required for the development of Coomb's positive hemolytic anemia, however, other susceptibility genes (or gene) from the NZB strain are absolutely required (Haraldsson, Theofilopoulos, Kono, unpublished data). Studies to determine the relationship between *Aia-1* and *Lbw2* are being pursued, as well as the relationships to other traits, such as hyperIgM, B cell hyperactivity and expansion of B1 cells identified in other crosses (22, 25) or interval congenic mice of C57BL/6 background (23, 24). Overall, the *Lbw2* interval is particularly fascinating because of the presence of a diversity of component phenotypes, of which some are unique among the lupus strains.

Lupus susceptibility loci in (MRL-*Fas*^{lpr} $\times$ B6-*Fas*^{lpr}) F_2 mice

Defects in the apoptosis-promoting Fas/FasL receptor-ligand pair of cell surface molecules, in *lpr* and *gld* mice, respectively, are associated with massive accumulations of CD4⁺CD8[−] (double negative) T cells, hypergammaglobulinemia, autoantibodies, and a spectrum of autoimmune manifestations that resemble lupus, systemic vasculitis, rheumatoid arthritis and Sjogrens syndrome (36, 37). Similar findings have also been observed in humans with the rare autoimmune lymphoproliferation syndrome (ALPS or Canale-Smith syndrome) caused by mutations in genes related to the Fas apoptosis pathway (Fas, FasL, caspase 10) (38). The mechanism by which Fas/FasL deficiency leads to these abnormalities appears primarily due to defective activation-induced cell death (AICD) of B and T cells in the periphery and the expansion of self-reactive cells (37). The expanded double-negative cell population, which is derived mainly from AICD-resistant CD8⁺ T cells that have down-regulated expression of CD8, is the hallmark of the *lpr* and *gld* mutations and enhances, but is not essential for the development of autoimmunity (39–41). Instead, evidence supports the requirement for both CD4⁺ T cells and B cells in this process (41–43), although, defective Fas-mediated apoptosis (AICD resistance) in only the B cell population may be sufficient for autoimmunity to develop (44).

Although defects in Fas-mediated apoptosis strongly predisposes to systemic autoimmunity, full expression of lupus disease clearly requires the presence of additional susceptibility genes. Thus, disease in non-autoimmune C57BL/6 mice homozygous for the *Fas^{lpr}* mutation is manifested by the late-onset production of autoantibodies, including both antinuclear and rheumatoid factor, but no further disease progression. In contrast, the lupus-predisposed MRL strain with either the *Fas^{lpr}* or *Fas^{gld}* mutation develops not only early onset autoantibodies, but severe GN, vasculitis, and arthritis that results in a 50% mortality around 5–6 months of age (37, 45). *Fas^{lpr}*-mediated autoimmune disease acceleration is not limited to the MRL background, but to other lupus-predisposed strains as well, including the NZB, NZW and BXSB (46).

To define the MRL background susceptibility genes, we examined a panel of (MRL × C57BL/6)*F₂*-*Fas^{lpr}* intercross mice for QTL to lymphoproliferation, IgG antidsDNA antibody production, and GN (47). Four major loci were initially identified, designated *Lmb1–4*, on chromosomes 4, 5, 7 and 10, respectively. All four QTL were linked to lymphoproliferation, three were also linked to antidsDNA antibodies (*Lmb1–3*) and one was also linked to GN (*Lmb4*) (Table 2). As expected, the susceptible QTL for most of these loci were derived from the MRL strain (*Lmb2–4*), however, one of the QTL, *Lmb1*, was linked to the nonautoimmune C57BL/6 strain. Despite this, the contribution of *Lmb1* to lymphoproliferation was comparable to the other QTL, with all QTL being additive, but with certain combinations associated with greater effects. *Lmb1* may contribute to the late onset and milder lymphoproliferation and antidsDNA autoantibodies observed in C57BL/6-*Fas^{lpr}* mice. These findings raise the possibility that mice considered nonautoimmune may also have susceptibility alleles, but that the number and specific combination of these alleles may not be sufficient to induce overt disease. In this regard we have sporadically observed elevated levels of antichromatin antibodies in some C57BL background mice (unpublished data).

Interestingly, although antidsDNA autoantibodies are considered pathogenic for GN, this trait did not map to *Lmb4*, the only locus significantly linked to GN. This suggested that perhaps *Lbm4* predisposed to a later stage of disease pathogenesis than autoantibody production, or possibly to the production of other

pathogenic autoantibody specificities. To explore the latter, we mapped in the (MRL × C57BL/6)*F₂*-*Fas^{lpr}* intercross mice, loci predisposing to autoantibodies to other nuclear antigens, Sm, IgG (rheumatoid factor, RF) and the endogenous retroviral glycoprotein, gp70 (Table 3) (Vidal, Kono, Izui, Theofilopoulos, unpublished data). Autoantibodies to ssDNA and chromatin were all linked to the same regions as the previously mapped antidsDNA response (*Lmb1–3*). This strongly suggests that generation of these antinuclear antibodies are causally related and share similar induction processes. This would be compatible with previous studies suggesting an evolution of response from antichromatin to anti-DNA (48). The related antihistone response also mapped to chromosomes 4 (*Lmb1*) and 5 (*Lmb2*), but also to the C57BL/6 MHC region (H-2^b). Antibodies to Sm mapped to *Lbw3* and also to the MHC, but in this case to the MRL background (H-2^k), which is consistent with the presence of anti-Sm in MRL-*Fas^{lpr}*, but not C57BL/6-*Fas^{lpr}* mice. Inheritance of the various IgG subclasses of anti-IgM RF was more complex with responses mapping to different sets of loci depending on the IgG subclass (Table 3). There was only a single common locus on chromosome 9 that was derived from the C57BL/6 strain. The finding that this and most of the loci linked to RF were from the C57BL/6 genome is consistent with the higher levels of RF found in this strain compared with MRL. The autoantibody response to gp70 (detected as gp70 immune complexes, gp70IC) strongly mapped to a MRL locus on chromosome 13 and to the C57BL/6 MHC region (H-2^b). The identical chromosome 13 interval was also linked to the production of gp70, which suggests that this may be the site of the endogenous retroviral gp70 gene. Moreover, the findings suggest that loss of tolerance from the *Fas^{lpr}* mutation combined with the optimal MHC for antigen presentation and availability of antigen may be sufficient to lead to the development of autoantibodies. It is interesting to speculate that some physical or expression characteristic of the gp70 may also enhance such susceptibility to self reactivity and autoantibody generation. Thus, a mechanism for predisposing to

Table 2 Loci predisposing to lymphoproliferation, GN and antidsDNA autoantibody levels in (MRL-*Fas^{lpr}* × B6-*Fas^{lpr}*)*F₂* mice

Chr	Name	Strain	Trait
4	<i>Lmb1</i>	B6	Lymphoproliferation, antidsDNA Ab
5	<i>Lmb2</i>	MRL	Lymphoproliferation, antidsDNA Ab
7	<i>Lmb3</i>	MRL	Lymphoproliferation, antidsDNA Ab
10	<i>Lmb4</i>	MRL	Lymphoproliferation, GN

lod > 4.3 (significant linkage) for all *Lmb* loci (47).

Table 3 Loci predisposing to autoantibody levels in (MRL-*Fas^{lpr}* × B6-*Fas^{lpr}*)*F₂* mice

Chr	Strain	Autoantibody specificity
1	B6	RF (IgG2a, IgG3*)
4	B6	dsDNA*, ssDNA*, chromatin, histones, RF (IgG1*, IgG2a)
5	MRL	dsDNA*, ssDNA*, chromatin*, histones*, RF (IgG2a*)
7	MRL	dsDNA, ssDNA*, chromatin, Sm
9	B6	RF (IgG1, IgG2a, IgG2b, IgG3)
13	MRL	gp70*, gp70 IC*
	B6	histones, RF (IgG1), gp70 IC*
17	MRL	Sm*

*lod > 4.3 (significant linkage); all others lod > 2.8 (suggestive linkage). RF = rheumatoid factor (IgM anti-IgG); gp70 IC = gp70 immune complexes (measure of antigp70 autoantibodies).

autoantibody production, at least in the context of a major susceptibility gene (Fas defect), may simply be increased levels of the self antigen. In the case of the *Lmb1-3* loci, which predispose to anti-DNA autoantibodies, the genetic defects might alter levels of DNA, perhaps through abnormal production or uptake of apoptotic bodies (49, 50).

The findings clearly show that autoantibody specificity is controlled by different sets of susceptibility loci, which strongly supports a major role for genetic background in addition to stochastic factors in this process (51). Moreover, the manner in which genetic influences affect autoantibody profiles is complex with some loci shared among the different specificities, possibly because they enhance immune responses or the antigen specificities are related, whereas, others are unique. The finding that autoantibodies to protein antigens, such as histones, Sm, and gp70, were linked to the MHC, but not to the same haplotype, appears more consistent with a mechanism involving repertoire shaping or peptide presentation by the MHC class II molecules, rather than an alternative possibility of different, but closely linked nonMHC genes. Interestingly, none of the autoantibody promoting loci overlapped the GN-linked *Lmb4*, a finding similar to some of the BWF₂ GN-linked *Lbw* loci. Several possible explanations for this are that the specific glomerulonephritic autoantigen(s) have not been identified, development of GN is not dependent on a specific autoantibody specificity, or *Lmb4* predisposes to GN by a mechanism unrelated to antibody specificity.

Several other groups, using different crosses, have also identified loci in the MRL strain predisposing to lupus traits, including *Lrdm1* and *Lrdm2*, located on chromosomes 7 and 12, respectively (52) and *Lprml-5*, on chromosomes 4, 3, 14, 5, and 16, respectively (53, 54).

Concluding remarks

Genome-wide scans of all major lupus-predisposed strains have provided a glimpse of the multiple loci and interactions involved in genetic susceptibility, as well as an essential foundation for defining the underlying alleles and pathogenic mechanisms. This line of investigation will certainly provide new insights into the pathogenesis of SLE and should lead to the development of novel approaches to disease management.

Note added in proof

Candidate genes for two different loci on chromosome 1 were reported after submission of this manuscript. The *Ifi202* gene, located in the *Nba* interval, was found to be abnormally elevated in NZB mice (55). Its function and how overexpression promotes autoimmunity are not known. Within the *Slelc* subinterval, a point mutation of *Cr2* was discovered in NZW and NZW/Aeg2410 mice (56). This introduces a new glycosylation site leading to reduced ligand binding and receptor-mediated cell signalling.

Acknowledgments

This is Publication number 13894-IMM from the Department of Immunology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037. The work of the authors reported herein was supported by National Institutes of Health Grants AR31203, AR39555, AG15061, AR42242, and ES08666.

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