Susceptibility Loci for Lupus: A Guiding Light from Murine Models?

Editorial

Systemic lupus erythematosus (SLE) is an autoimmune disease in which autoantibodies have the potential to damage multiple organ systems and cause diverse clinical manifestations (1). A hallmark of the disease is the production of IgG antibodies directed to nuclear constituents (antinuclear antibodies). There is considerable evidence that the development of SLE has a strong genetic basis (reviewed in references 2 and 3). For example, studies of families with an affected member suggest that a sibling has a 20-fold increased risk of developing SLE compared with the general population. In addition, the concordance rate in monozygotic twins has been estimated to be 20–30% compared with $\sim$ 2% in dizygotic twins. The potential role of specific genes in SLE has been mostly investigated by association studies of candidate genes or gene complexes, especially the major histocompatibility complex (MHC). However, over the last several years, the availability of easy to use maps of genetic markers that cover the entire mouse and human genomes has revolutionized the study of genetic predisposition. These markers (microsatellites) can be used to map the chromosomal positions of genetic loci linked with a disease, therefore identifying regions of the genome that contain disease-susceptibility genes. The positions of contributing genes are isolated on the basis of location and not on the basis of any known function. Genome-wide scans for loci linked with type 1 diabetes (reviewed in reference 4) and multiple sclerosis (reviewed in reference 5) in affected sibpairs have been reported. A paper in this issue by Tsao and colleagues (6) marks a diversion from previous association studies in SLE and is the first to report the results of a (directed) linkage analysis of sibpairs affected with this disease.

SLE, like other autoimmune diseases, is a complex polygenic trait with contributions from the MHC and multiple non-MHC genes. In contrast to single-gene diseases, such as cystic fibrosis or Huntington's disease, the identification of etiologic mutations in complex traits has progressed slowly, caused primarily by the small increased risk of disease (i.e., penetrance) from each of the multiple contributing genes. Etiologic alleles in complex polygenic traits determine disease susceptibility and no particular gene is necessary or sufficient for disease expression. Even in the presence of a full set of susceptibility alleles at multiple loci, overt disease does not always develop (incomplete penetrance). There are additional factors that complicate the genetic analysis of a disease like SLE. For example, different combinations of genes, whether in different ethnic groups or even in the same family (or lupus-prone murine strain), may result in the identical disease phenotype (genetic heterogeneity). In addition, because most of these susceptibility alleles are unlikely to represent mutations with severe functional effects nor cause problems by themselves, they have not been selected against over many generations. Therefore, these alleles may be relatively common in the general population.

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Rodent models of disease have contributed greatly to understanding the immunopathogenesis of different autoimmune diseases, including SLE, type 1 diabetes, and multiple sclerosis (reviewed in 1–3). Theoretically, these models also could be remarkably helpful guides to dissect the genetic basis of the corresponding human disease. The use of animal models offers a number of potential advantages compared with direct genetic studies of patients. For example, directed breeding of backcross (i.e., F1 mice bred to one of the parental strains) or F2 intercross mice (i.e., F1 mice bred to each other) allows the generation of large numbers of offspring with and without disease adequate to map markers that cover the entire mouse genome. To conduct a genome-wide scan in humans, several hundred sibpairs would probably be necessary to document linkage for many of the contributing loci, and the collection and accurate phenotyping of this number of families is a major undertaking. Furthermore, the disease phenotype among mice in each cross is much more uniform compared to the relatively heterogeneous disease expression in patients. Especially in SLE, clinical manifestations and autoantibody production can be extremely diverse and variable, which is in part genetically based, and this variability can confound genetic studies. The study of animal models also provides an opportunity to control environmental exposures, and development of disease in experimental animal crosses is considered to be solely a reflection of the genes inherited.

As a guide to genetic predisposition in human disease, the results from animal models could be useful in two major ways. In the first, a mouse chromosomal region containing a susceptibility gene will generally have a homologous (syntenic) region in the human genome which can be tested in a linkage study of families. Thus, a linkage analysis could be directed to particular genomic regions rather than involve several hundred markers to complete a genome-wide scan. This approach was used in the present study by Tsao and colleagues (6). In addition, animal models could be useful once the actual genes contributing to disease are identified. The same gene, other genes in the same biological pathway, and perhaps genes in other related pathways could be tested for either linkage or association with the human disease. Unfortunately, although multiple loci have been mapped in linkage analyses of diabetes in NOD mice and lupus in New Zealand mice, none of the non-MHC susceptibility genes have been identified to date (2, 3).

New Zealand mice, particularly hybrids of New Zealand black (NZB) and New Zealand white (NZW) mice, are one of the best-studied models of lupus nephritis and pathogenic IgG anti-DNA autoantibody production. These mice have been subject to genome-wide linkage studies in an attempt to map the chromosomal positions of disease susceptibility genes. Data from seven independent studies have mapped at least 12 loci (in addition to the MHC) linked with nephritis and/or autoantibody production (reviewed in reference 3). When different mapping studies are compared, a locus on distal murine chromosome 1 has demonstrated remarkably consistent evidence for linkage. An NZB locus on distal chromosome 1, termed *Nba2* for *New Zealand black autoimmunity 2*, appeared to be the most important non-MHC locus linked to nephritis and death in three different backcrosses, and was the

only genetic contribution that spanned different genetic backgrounds (7, 8; reviewed in reference 3). A locus at a similar position on chromosome 1, designated *Sle1*, was mapped in NZM backcross mice (NZM is a recombinant inbred of NZB and NZW strains) (9). This locus appears to be NZW in origin, whereas *Nba2* is an NZB locus. Finally, a distal chromosome 1 locus, designated *Lbw7*, was found to be linked with anti-chromatin production in (NZB × NZW)F2 intercross mice (10).

Tsao and colleagues (6) probably began their directed linkage analysis with mixed feelings regarding possible success. On one hand, the relative importance of the susceptibility locus on distal murine chromosome 1 (encompassing *Nba2/Sle1/Lbw7*) and its consistent contribution to murine lupus made it a likely guide for directed screening of syntenic regions in human patients (8). Furthermore, there has been a recent report of positive findings in a directed linkage analysis of patients with multiple sclerosis guided by syntenic loci mapped in murine experimental autoimmune encephalomyelitis (EAE) (11). In contrast, despite genome-wide scans that have identified at least 13 non-MHC *Idd* loci in the NOD model of type 1 diabetes and about 10 *IDDM* loci in the human disease, only one non-MHC locus may correspond in both diseases (2, 4).

The report in this issue describes the analysis of seven chromosome 1 microsatellite markers in 52 affected sibpairs. The statistical support for linkage with markers at 1q41-42 is remarkable when one considers the small number of affected sibpairs studied, the racial heterogeneity of the families, and that there was no attempt to subset the analysis based on phenotype (i.e., individuals were counted as affected if they met the ACR criteria for classification of SLE). As the authors suggest, such positive findings may indicate that the penetrance of this locus is remarkably strong and that it may predispose to disease across different ethnic groups. If this reasoning is correct, linkage with markers in this chromosomal region should readily be replicated when a separate set of lupus families is studied.

However, there should also be concern that an initial mapping in a complex trait reflects false positive findings. In the current study, the markers at 1q41-42 found to be linked with disease may be 5 centiMorgans beyond the distal boundary (95% confidence interval) for the murine chromosomal interval containing *Nba2* and are probably beyond the distal border for *Sle1* as well (Vyse, T.J., S.J. Rozzo, C.G. Drake, S. Izui, and B.L. Kotzin, manuscript submitted for publication). If true, this human locus may not be in a region syntenic to the murine susceptibility locus, and linkage in the current human study would therefore represent quite a fortuitous finding. It is emphasized that the current study had limited power to exclude a susceptibility locus more proximal on human chromosome 1; it may require several hundred to one thousand sibpairs or transmission disequilibrium testing to document linkage at this position (4). Because the current study used a directed mapping approach, it did not involve the multiple hypothesis testing of a genome-wide scan, and therefore, the recommended stringent statistical thresholds to claim linkage (12) in such a study do not apply. However, if the initial hypothesis regarding synteny is not correct, the proper statistical threshold to use may need to be more stringent than if the hypothesis is correct. Future analysis of a separate group of lupus families will be necessary to confirm whether there is a locus at 1q41-42 with unusually strong contributions to disease risk.

Having identified a genetic locus in linkage with disease, the next step is to identify the disease-susceptibility gene at that locus. The chromosomal intervals initially mapped in a linkage analysis of murine crosses or affected sibpairs are usually 10–20 centiMorgans, and therefore may contain up to 500 genes, most of which are unknown. Choosing candidate genes based on the function of known genes without narrowing the interval is a risky but hard to resist approach. Attractive candidates for the murine locus on distal chromosome 1 include the Fc gamma receptor genes, *Fcgr2* and *Fcgr3*. Studies in human SLE have also demonstrated a significant association for a particular FcγRIIA allele with lupus nephritis (reviewed in reference 3). As discussed (6), this gene appears to be outside of the interval mapped in the current study. Ongoing studies in murine lupus are focusing on reducing the intervals containing the chromosome 1 and other susceptibility genes so that candidate gene approaches will be more likely to succeed and/or positional cloning techniques can be used to identify the etiologic allele. Similar goals will follow initial linkage studies in human SLE, although the strategies employed to accomplish this work, such as transmission disequilibrium testing, will be quite different compared to those used in murine disease.

In summary, the linkage study reported in this issue may be an important step toward identifying a susceptibility gene on chromosome 1 in human SLE. The genes that predispose to SLE and other autoimmune diseases must, *ipso facto*, be related to key events in pathogenesis. Their identification will almost certainly provide important new insight into the breakdown of immunological self tolerance and the cause of autoimmune disease.

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References

1. Kotzin, B.L. 1996. Systemic lupus erythematosus. *Cell.* 85:303–306.
2. Vyse, T.J., and J.A. Todd. 1996. Genetic analysis of autoimmune disease. *Cell.* 85:311–318.
3. Vyse, T.J., and B.L. Kotzin. 1996. Genetic basis of systemic lupus erythematosus. *Curr. Opin. Immunol.* 8:843–851.
4. Todd, J.A., and M. Farrall. 1996. Panning for gold: genome-wide scanning for linkage in type 1 diabetes. *Hum. Mol. Gen.* 5:1443–1448.
5. Bell, J.I., and M. Lathrop. 1996. Multiple loci for multiple scerosis. *Nat. Genet.* 13:377–378.
6. Tsao, B.P., R.M. Cantor, K.C. Kalunian, C.-J. Chen, H. Badsha, R. Singh, D.J. Wallace, R.C. Kritidou, S.-L. Chen, N. Shen, Y.W. Song, D.A. Isenberg, C.-L. Yu, B.H. Hahn, and J.I. Rotter. 1997. Evidence for linkage of a candidate chromosome 1 region to human systemic lupus erythematosus (SLE). *J. Clin. Invest.* 99:725–731.
7. Drake, C.G., S.J. Rozzo, H.F. Hirrschfeld, N.P. Smarnworawong, E. Palmer, and B.L. Kotzin. 1995. Analysis of the New Zealand black contribution to lupus-like renal disease: multiple genes that operate in a threshold manner. *J. Immunol.* 154:2441–2447.
8. Rozzo, S.J., T.J. Vyse, C.G. Drake, and B.L. Kotzin. 1996. Effect of genetic background on the contribution of New Zealand black loci to autoimmune lupus nephritis. *Proc. Natl. Acad. Sci. USA.* 93:15164–15168.
9. Morel, L., U.H. Rudofsky, J.A. Longmate, J. Schiffenbauer, and E.K. Wakeland. 1994. Polygenic control of susceptibility to murine systemic lupus erythematosus. *Immunity.* 1:219–229.
10. Kono, D.H., R.W. Burlingame, D.G. Owens, A. Kuramochi, R.S. Balderas, D. Balomenos, and A.N. Theofilopoulos. 1994. Lupus susceptibility loci in New Zealand mice. *Proc. Natl. Acad. Sci. USA.* 91:10168–10172.
11. Kuokkanen, S., M. Sundvall, J.D. Terwilliger, P.J. Tienari, J. Wikstrom, R. Holmdahl, U. Pettersson, and L. Peltonen. 1996. A putative vulnerability locus to multiple sclerosis maps to 5p14-p12 in a region syntenic to the murine locus *Eae2*. *Nat. Genet.* 13:477–480.
12. Lander, E., and L. Kruglyak. 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat. Genet.* 11:241–247.