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Genetics

Genetic analysis of diabetes and insulitis in an interspecific cross of the nonobese diabetic mouse with *Mus spretus*

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ABSTRACT **The nonobese diabetic (NOD) mouse is a widely used model for genetic studies of insulin-dependent diabetes mellitus due to the similarities between the murine and human diseases. To aid in the localization and identification of diabetes-related susceptibility genes, we have constructed an interspecific backcross between NOD and *Mus spretus* (SEG/Pas) mice. Although no diabetic animals were observed in the first backcross generation of (SEG/Pas × NOD) × NOD (BC_1), the incidence of insulitis (lymphocyte infiltration of the islets of Langerhans) exceeded 20% after injections of cyclophosphamide, a treatment that provokes an acute form of diabetes in NOD mice. Insulitis, a prediabetic condition, is a useful phenotype in studies of diabetes susceptibility. In the second backcross (BC_2) generation, 8% of the animals became diabetic and 76% were found to have insulitis. Genetic mapping studies in the BC_2 families confirmed the importance of the major histocompatibility complex region on the severity of insulitis and suggested that additional susceptibility loci were linked to markers on mouse chromosomes 3, 6, and 15. *Mus spretus* crosses have been an important tool in recent advances in murine genetics, and our results extend their usefulness to the study of a multifactorial disease.**

Insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease with multifactorial etiology in which both heterogeneous genetic factors and environmental influences play a role (1). The selective destruction of insulin-secreting cells, a constitutive feature of IDDM, is due to an immune reaction that predominately involves autoreactive T cells and shows a strong association with class II major histocompatibility complex (MHC) alleles (2–8). The disease has similar features in the nonobese diabetic (NOD) mouse, which has proven to be a useful animal model for understanding the genetic susceptibility to IDDM (9). In NOD mice, spontaneous diabetes occurs predominately in female animals 10–12 weeks of age or older. The onset of diabetes is preceded by lymphocyte infiltration into the islets of Langerhans of the pancreas (insulitis), which can first be observed at 3–4 weeks of age (10). An acute form of diabetes can be induced in both NOD males and females by injection of cyclophosphamide (11).

Previous breeding studies have suggested that several genes are involved in diabetes susceptibility in NOD mice (6–8). Genes in the MHC region are implicated in the etiology of both the murine and human diseases (12–14). The apparent conservation of the MHC-linked susceptibility gene in humans and mice suggests that other non-MHC diabetes susceptibility genes may also be conserved in the two species (15). Genetic linkage studies using crosses of NOD mice with other inbred laboratory strains that are resistant to diabetes have shown linkage of diabetes susceptibility or insulitis to markers on mouse chromosomes 1, 3, 9, and 11 (6, 16–18).

In the past, genetic mapping studies in the mouse have been hampered by the restricted occurrence of restriction fragment length polymorphism allelic differences between inbred laboratory strains. Recently, minisatellite and microsatellite tandem repeats have been shown to be highly polymorphic in laboratory mice and to provide a useful source of genetic markers for linkage studies (18, 19). Microsatellite markers have been used for the localization of some of the genes responsible for diabetes susceptibility and insulitis in the NOD mouse (18). Although microsatellite marker coverage of the mouse genome is increasingly dense, sequence similarities between inbred laboratory strains will continue to slow attempts to obtain a more precise localization based on the analysis of recombination events.

The high intrinsic levels of sequence variation between *Mus spretus* and classical inbred strains has been of major importance in the rapid progress made in establishing detailed genetic maps of the mouse (20). Theoretically, interspecific crosses of NOD mice with *Mus spretus* mice should be advantageous for the initial detection of linkage and especially for the subsequent refining of the localization of genes responsible for the disease phenotype based on the high density of informative markers available throughout the genome. Such crosses allow the use of both PCR-based microsatellite markers and restriction fragment length polymorphisms detected by hybridization. Crosses between NOD and *Mus spretus* mice are equally of interest because their characterization may lead to the identification of additional susceptibility loci that are invariant between NOD mice and other inbred laboratory strains. Although interspecific crosses have been applied to the genetic study of monogenetic traits (21), their usefulness in the study of complex traits such as diabetes has yet to be established.

Here, we report on the investigation of cyclophosphamide-induced diabetes and insulitis in first and second backcross (BC_1 and BC_2, respectively) cohorts of *Mus spretus* × NOD mice designed to evaluate this experimental system for genetic mapping studies. Linkage studies for insulitis were performed in up to 326 BC_2 animals in 19 families with a set of 109 mapped minisatellite and microsatellite markers covering most of the mouse genome.

MATERIALS AND METHODS

Breeding Schedules. NOD mice were obtained from E. Leiter (The Jackson Laboratory) in December 1988 when they were at generation F_{48}. Female NOD mice were mated with males of the inbred *Mus spretus* strain SEG/Pas derived at the Institut Pasteur, then at generation F_{14}. F_1 females were

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Abbreviations: NOD, nonobese diabetic; BC, backcross; MHC, major histocompatibility complex.

subsequently backcrossed to NOD/Lt males and the resulting BC_1 animals were typed for H-2 (see below). A subset of 26 animals consisting of 15 NOD H-2 homozygotes and 11 NOD H-2 heterozygotes were placed in separate cages where they were mated to individual NOD/Lt males to provide BC_2 families. Progeny were tested in BC_2 families descended from 19 BC_1 animals, of which 8 were NOD H-2 heterozygotes. Seven of the BC_1 female progenitors failed to breed probably due to sterility problems linked to the use of the interspecies cross.

A total of 93 BC_1 animals and 326 BC_2 animals were analyzed for their diabetes phenotype (see below). Ninety-five BC_1 animals were included in a mapping panel; 47 of these animals have not been tested for diabetes.

H-2 Typing. Mice were individually bled retroorbitally into heparinized tubes. Blood lymphocytes were isolated and incubated with biotinylated 10.3.6 (anti-I-A nod) (22) or 14.4.4 (anti-I-E) (23) monoclonal antibodies, washed, and then incubated with streptavidin R-PE (Caltag). Immunofluorescence staining analysis was performed using a FACScan (Becton Dickinson) equipped with a 488-μm argon laser and linked to a Hewlett–Packard computer.

Cyclophosphamide Treatment and Diabetes. Eight-week-old BC_1 and BC_2 animals received two intraperitoneal injections of cyclophosphamide (Endoxan-Astra, Laboratories Lucien, Colombes, France; 200 mg/kg) at an interval of 14 days. Animals were monitored for glycosuria (Glucotest; Boehringer Mannheim) every 3 days and assessed for glycemia 14, 21, and 28 days after the initial injection by using test strips and a colorimetric assay (Haemogluctest and Reflolux F; Boehringer Mannheim). Diabetes was diagnosed when permanent fasting glycemia >3 g/liter was observed.

Histopathology. Pancreases were collected 14 days after the second cyclophosphamide injection and fixed in Bouin's solution. Paraffin sections (4 μm) were stained with hematoxylin/eosin. Insulitis was evaluated by the method of Wicker *et al.* (8). A histology grade was assigned on a scale of 1–6 based on the percentage of islets showing a lymphoid infiltrate within or around the islets. The following classification system was used: 1, no abnormal lymphocyte accumulation; 2, periislet infiltration, no insulitis; 3, mild insulitis in some islets but with no reduction in islet mass; 4, extensive insulitis with significant islet mass remaining; 5, extensive insulitis with significant reduction in islet mass; 6, extensive insulitis with only residual islets remaining.

Histology scores were obtained in 76 BC_1 and 326 BC_2 animals after cyclophosphamide treatment. Histology was also obtained on an additional 17 BC_1 animals at 9 months of age or older that had not been treated by cyclophosphamide. Eleven SEG/Pas control animals were also treated with cyclophosphamide and analyzed histologically.

DNA Extraction. Splenocyte suspensions were obtained by teasing apart the spleen into sterile ice-cold phosphate-buffered saline (PBS), centrifuged, and resuspended in 2 ml of PBS, and then an equal volume of 2× lysis buffer (24) was added. After overnight incubation at 55°C, the DNA was processed by standard procedures (24).

Markers and Genetic Map. A combination of microsatellite and minisatellite markers were characterized for linkage studies in BC_2 animals. Primer pairs for the characterization of the microsatellite markers were obtained from the literature (25–29). PCR conditions for genotype determination were as described (30). The chromosome assignments and localizations of the microsatellite markers were verified by linkage in the BC_1 or BC_2 panels. Minisatellite markers were obtained by characterization of eight human variable number of tandem repeats (VNTR) probes (pYNZ2, pEFD134.7, pYNH24, pAW101, pINS310, pYNZ22, pUCJ, and pMV9.17) and one other probe (pSP.2.5.EI) that were shown to detect multiple loci (fingerprint patterns) under nonstringent hybridization conditions as described (19). Initially, a genetic map of the minisatellite markers was constructed using the LINKAGE programs (31) from data obtained on the mapping panel of 95 BC_1 animals. The BC_1 panel was characterized with restriction fragment length polymorphism markers with known localizations to anchor the map (data not shown). Confirmation of the genetic map and marker order was obtained in the BC_2 animals. Previously published microsatellite markers for certain chromosome regions were also characterized in the BC_2 panel permitting additional assignments of minisatellite markers. When a potentially significant linkage was found with minisatellite bands, we examined microsatellite markers from the same region to confirm the result. Seventy-four mapped minisatellite and 35 mapped microsatellite markers that had been characterized in at least 175 BC_2 animals were selected for analysis of linkage to histology grade.

Genetic Analysis. Estimates for the total phenotypic variance V_t and the within-family sib correlation V_c in the BC_2 were obtained using a maximum likelihood method that takes into account unequal family sizes (32). The within-family covariance of a phenotypic trait can be shown to be equal to $V_c = V_g/3$, where V_g is the genetic variance in the BC_2 cohort. Therefore, the proportion of the total phenotypic variance due to genetic factors in the BC_2 can be estimated as $V_g = 3V_c/V_t$.

A subset of minisatellite bands that had been mapped in the BC_1 panel and previously published microsatellite markers (25–29) were first screened for potential linkage to the histology grades by using a nonparametric statistic. The means of the sex-adjusted histology scores were compared in homozygotes and heterozygotes at each locus using the following statistic: $F = (m_{nn} - m_{ns})^2/V$. Here m_{nn} and m_{sn} are the means in NOD/NOD homozygotes and NOD/SEG heterozygotes, respectively, and V is the pooled variance estimate. To account for phenotypic correlations within families and deviations from normality, an empirical significance level was obtained by a Monte Carlo method. The F statistics were evaluated in 50,000 simulated replicates under the assumption of no linkage between the marker and phenotype. For the simulations, the genotype status of the BC_1 parents and the histology grades in the BC_2 animals were held fixed, and marker genotypes were randomly assigned to members of the BC_2 generation conditional on the parental genotypes. The significance was measured as the frequency in which F reached or exceeded the observed value in 50,000 replicates. Further tests of linkage were made when the empirical significance of the F static was 0.01 or less. These tests were performed using a mixed model (33, 34) that includes a linked major gene with residual correlation due to other genetic factors. Empirical P values were also calculated for the mixed model by simulation. As is traditional for genetic linkage studies, we report P values without adjustment for the number of marker loci that were tested.

RESULTS

Histopathology and Diabetes in BC_1 and BC_2 Cohorts. In the BC_1 generation, we found no diabetes among 73 cyclophosphamide-treated animals. Histological examination revealed that 48 (66%) of the cyclophosphamide-treated animals showed evidence of insulitis (histology grade 3 or greater), and 16 (22%) had extensive insulitis (histology grade 4 or greater). In comparison, all 11 SEG/Pas control animals showed histology scores of 1 or 2 after cyclophosphamide treatment. We also undertook histological examinations in 17 untreated BC_1 females at 13 weeks of age or older; 13 (76%) exhibited evidence of insulitis and 8 (47%) had extensive insulitis. These results are detailed in Fig. 1.

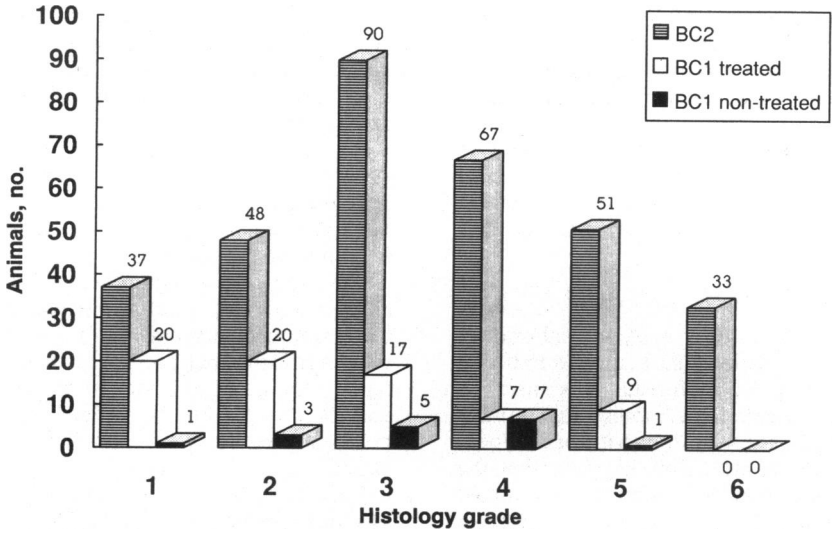

FIG. 1. Distribution of histology grades in BC_2 and BC_1 animals treated with cyclophosphamide at 8 weeks of age and untreated BC_1 animals at 36 weeks of age. Numbers above the bars indicate the number of animals in each histology class.

Because of the very low incidence of diabetes in the BC_1 animals, we decided to construct a BC_2 cohort for further study. As described above, a total of 326 progeny were produced by 19 BC_1 animals with sibship sizes ranging from 4 to 45 (total of all litters). Seven of the BC_1 parents were heterozygous at the MHC loci, and 33 of their BC_2 progeny were MHC heterozygotes. Twenty-six (8%) of the BC_2 animals exhibited cyclophosphamide-induced diabetes, and insulitis was observed in 241 (76%), with extensive insulitis in 151 (46%). The histology scores were symmetrically distributed overall with a mean of 3.6 (Fig. 1). On average, the histology grades differed in male and female animals ($P = 0.003$), and the variation between families was highly significant ($P < 0.0001$) (Fig. 2). The correlation between offspring within the same family was estimated as 0.19, and 57% of the variance of the histology in the BC_2 cohort was attributed to genetic factors (estimated as described above).

MHC. Tumor necrosis factor (TNF) maps to the MHC region on mouse chromosome 17 (35), and a microsatellite marker of the *Tnf-α* gene (25) detects allelic variation between NOD and SEG mice. We used *Tnf-α* to deduce MHC genotypes in the BC_2 animals. Although our criteria for further evaluation of linkage was not met in the initial screen ($P = 0.09$), the distribution of histology showed considerable variation by genotype. Indeed, of the 33 NOD/SEG MHC heterozygotes observed, none exhibited extensive insulitis with significant reduction of islet cell mass (histology grade of 5 or greater), whereas 83 of 286 homozygote animals (29%) were in this category. However, linkage to the MHC was not significant when all histopathology grades were considered ($P > 0.1$). This suggests that MHC status is a necessary factor for severe insulitis but has less influence on the degree of islet infiltration in milder forms of the condition.

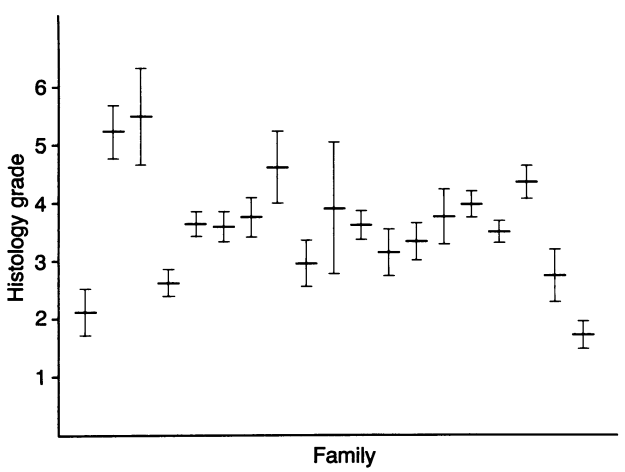

FIG. 2. Histology grades (mean ± SEM) in animals for 19 BC_2 families.

Linkage Analysis with Other Markers. Linkage to other markers was evaluated in two stages. (*i*) Chromosome regions that showed evidence of linkage were selected from results obtained from the initial 175 randomly characterized BC_2 animals tested. (*ii*) Markers were characterized in selected regions using the remaining panel of up to 326 animals. The genetic map spanned ≈1400 centimorgans and included markers on all autosomal chromosomes and the X chromosome.

The statistics for groups of markers on chromosomes 3 and 6 and a single marker on chromosome 15 (*Krt-2*) exceeded our initial criterion for further evaluation of linkage (estimated *P* values < 0.01). On chromosome 3, the marker showing the most significant correlation to histology was *D3NDS2*, and on chromosome 6, it was *DODNS4*. In all three instances, NOD/NOD homozygotes exhibited higher mean histology scores than NOD/SEG heterozygotes. Test statistics and estimates of the genetic variances attributed to the linkage markers with the highest correlations on these three chromosomes are given in Table 1. The recombination estimates between the marker and the hypothesized trait loci are zero on each chromosome, but the estimates have large standard error because of confounding with other variables in the genetic model (results not shown). We estimated that 32% of the genetic variance might be attributed to linkage of these markers by assuming additive effects at different loci. The

Table 1. Linkage and variance analysis

Chromosome	Marker	V_m/V_g	Test statistic	*P* value
3	*D3Nds2*	0.04	7.8	0.0080
6	*D6Nds1* (*DODNS4*)	0.20	12.0	0.0004
15	*D15Nds6* (*Krt-2*)	0.08	8.7	0.0057

Likelihood ratio test statistics for linkage, empirical *P* values from simulations (50,000 replicates), and estimates of the variance attributed to a linked major gene (V_m) compared to the total genetic variance (V_g) for markers on chromosomes 3, 6, and 15 are shown.

strongest effect was associated with *DONDS4*, which accounted for 20% of the genetic variance in this analysis (Table 1). Note that a replicate cross is required to confirm these results in the absence of a significance equivalent to a logarithm of odds score of 3 or more.

The remaining genetic variance may be accounted for in part by linkage to other marker loci for which the initial criteria of $P < 0.01$ criterion was not attained. Markers on chromosomes 16 and 12 gave $P < 0.05$ in these tests.

DISCUSSION

Initially, the incidence of diabetes in crosses with laboratory strains of mice led to the suggestion that a small number of recessive genes could be responsible for diabetes susceptibility in NOD (7). Recently, genetic mapping studies have demonstrated the existence of several loci with partial penetrance in heterozygotes (6, 16–18). This suggests that the number of susceptibility loci may be larger than estimated from a purely recessive model. The low incidence of diabetes in the BC_1 and BC_2 generations in this study demonstrates that alleles segregating at a large number of loci are implicated in insulitis and diabetes susceptibility in our interspecific cross. This has implications for the study of the human disease, where we may expect genetic susceptibility to be equally complex.

An interesting feature of our study design is the use of the BC_2 families for linkage investigations. The complexity of this design is intermediate between those based on BC_1 or F_2 cohorts and matings in outbred human populations. Therefore, it is important for planning of genetic studies in humans to evaluate the effectiveness of the BC_2 generation for detection of linkage. Our results suggests that genes on chromosomes 3, 6, and 15 are associated with the severity of insulitis in a NOD × SEG BC_2, but further confirmation in a replicate cross will be needed to confirm these linkages with the stringent criterion of a logarithm of odds score >3, despite the large number of animals used in the present study. Linkage analysis with several different approaches, including a classical mixed model (33), a class D regressive model (34), and a genetic model based on a polychotomous discrete trait led to similar conclusions (results not shown). This demonstrates that the results are not sensitive to the assumptions of a particular model.

Our study was limited to cyclophosphamide-induced insulitis and diabetes. The mechanism by which cyclophosphamide induces an acute form of disease in NOD mice is unknown, and additional genes may be important in the development of spontaneous forms of NOD diabetes. However, the possible identification of a similar region that contains susceptibility loci for the spontaneous and cyclophosphamide-induced forms of disease in two different crosses (chromosome 3; see below) suggests that the latter is a useful phenotype for genetic mapping studies.

This study demonstrates the usefulness of interspecific crosses involving *Mus spretus* to localize genes implicated in susceptibility to a complex disease. Theoretically, the *Mus spretus* system presents advantages over crosses involving standard inbred mouse strains for high-resolution linkage mapping due to the high degree of sequence variation found in this species compared to classical inbred laboratory strains of mice.

Our study was undertaken with a combination of minisatellite and microsatellite markers. We have described (36) the wide distribution in the murine genome of minisatellite bands detected by human variable number of tandem repeats markers and other probes under nonstringent hybridization conditions. Here, we have shown that mapped sets of minisatellite bands, combined with single locus probes, can serve as useful markers for the localization of genes in murine disease. The use of a panel of BC_1 progeny from a SEG × NOD intercross has enabled a large set of minisatellite markers identifying SEG-derived polymorphisms to be mapped with much greater precision than was possible from using recombinant inbred lines (19).

Possible linkage to the region of *DONDS4* on chromosome 6 and *Krt-2* on chromosome 15 are features of our results not described in previous linkage studies with different crosses. Several candidate genes for diabetes-susceptibility loci are known to lie on chromosome 6. These include the *Igk, Tcrb*, and islet amyloid polypeptide loci (37). The insulin loci *Ins-1* and *Ins-3* (one of which is a pseudogene) have been mapped to chromosomes 6 and 15 (37). Other potential candidates that have been mapped to the distal half of chromosome 15 include the gene for tumor transplantation antigens *D15Pas1* and *D15Pas3* (38). As stated above, confirmation of the results in a replicate experiment is needed before any of these candidates can be pursued.

Linkage of diabetes susceptibility and insulitis to a region of chromosome 3 containing *Tshb*, which is ≈26 centimorgans from *D3NDS2*, has been obtained in a NOD × C57/Bl10 BC_1 cohort (18). Several marker loci in this region, including *Tshb* and markers flanking it, exhibited evidence favoring linkage in our cross (results not shown). It is interesting that in NOD × C57/Bl10 mice, the strongest evidence for linkage on chromosome 3 was found in a region flanked by *Il-2* and *Tshb*, whereas we observed the strongest linkage at *D3NDS2*, distal to *Tshb*. These differences could be due to statistical variation or to the influence of different susceptibility loci on chromosome 3 in the two systems.

The NOD × SEG BC_2 cohort exhibits no evidence for linkage in the region of chromosome 1 that was found linked to insulitis in NOD × C57/Bl10 (16) and NOD × C57/Bl6 (17) mice or to chromosome 9 as described in NOD × NON mice (6). Todd *et al.* (18) have also found linkage to the *Acrb–Mpo* region of chromosome 11 in their cross, but principally in diabetic animals with an age-of-onset of <143 days. This linkage is not observed in our data.

Our experimental design allows comparison of the relative effects of MHC and other loci on insulitis. Although linkage to the MHC region was not significant in statistical tests in which residual familial correlation due to other genes were taken into account, the effect appeared stronger than other linkages in animals with diabetes or significant reduction of islet cell mass. When the 83 BC_2 animals with a histology grade of 5 or greater were examined, we found that none were MHC heterozygotes compared to 33 (16%) animals in the lower histology grades. For the loci given in Table 1, the equivalent heterozygote frequencies were (*i*) 4% vs. 10% for *DONDS4*; (*ii*) 6% vs. 18% for *Krt-2*; and (*iii*) 19% vs. 32% for *D3NDS2*. Thus, the effect of MHC may be more significant than those of other loci for severe insulitis and diabetes. In another cross, diabetes has been found in only a small proportion of animals that were heterozygous for the MHC region (39).

Initially, we adopted a BC_2 analysis because of the absence of cyclophosphamide-induced diabetes in the BC_1 generation. Histological examination showed that extensive insulitis (grade 5 or greater) occurred in only 21% of BC_1 animals compared to 47% of BC_2 animals after cyclophosphamide treatment at 8 weeks of age. Twenty-six animals became diabetic in the BC_2 generation, which confirmed that diabetes susceptibility factors were segregating in this cross. Although this number of diabetic animals allowed us to confirm the importance of the MHC region, it was insufficient to allow wide-scale genetic mapping studies. Therefore, we chose to examine histopathology as a phenotypic trait, due to its close relationship with diabetes and its distribution in the BC_2.

For linkage analysis, the study of BC_2 families introduces complexities that are not found in traditional BC_1 or F_2

designs. A large number of families are needed because of the frequency of homozygosity (50%) in the backcross parents. Since several loci determine a polygenic trait such as insulitis, it is necessary to account for phenotypic variation due to the possible segregation of different subsets of the loci in each family. A highly significant phenotype correlation was found within families; to account for this, we included residual polygenic effects in the linkage analysis. We estimated that 57% of the variance in histology was due to additive genetic effects and that the genetic correlation between offspring within the same family was 0.19.

We estimated that linkage to chromosome 6 could account for 20% of the genetic variance within the BC_2 generation and that linkages to chromosomes 3 and 15 could contribute an additional 12% of the genetic variance. The remaining proportion of the additive genetic variance could be due to a large number of minor genes or major effects that have not been detected with the markers and analyses performed here. Although our linkage map spans >1400 centimorgans, several areas of the genome, such as the proximal X chromosome and regions of chromosome 19, are not yet fully covered.

If our results are confirmed, it should be possible to exploit the interspecific cross for high resolution-linkage mapping of individual disease-susceptibility genes. BC_1 individuals can be selected based on PCR typing of markers linked to high histology grades to produce informative BC_2 families segregating for one of the major susceptibility loci that have been detected in this study or combinations of these loci to investigate their possible interaction. Also it will be possible to construct congenic lines segregating in NOD mice only for the portions of chromosomes 3, 6, or 15 containing the disease-susceptibility loci. The large degree of sequence variation between NOD and SEG mice within the selected region should allow a precise localization of a susceptibility locus to be obtained from the analysis of recombination events. In turn, this localization could lead to physical mapping studies or the study of a candidate gene and eventually the identification of the susceptibility gene.

Note Added in Proof. A full list of the markers used, their distribution, and the *P* values obtained has not been included for reasons of space but is available from the authors on demand.

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1. Castano, L. & Eisenbarth, G. S. (1990) *Annu. Rev. Immunol.* **8**, 647–667.
2. Bendelac, A., Carnaud, C., Boitard, D. & Bach, J. F. (1987) *J. Exp. Med.* **166**, 823–832.
3. Bendelac, A., Boitard, C., Bedossa, P., Bazin, H., Bach, J. F. & Carnaud, C. (1988) *J. Immunol.* **141**, 2625–2628.
4. Wicker, L. S., Miller, B. J. & Mullen, Y. (1986) *Diabetes* **35**, 855–860.
5. Miller, B. J., Appel, M. C., O'Neil, J. J. & Wicker, L. S. (1988) *J. Immunol.* **140**, 52–58.
6. Prochazka, M., Leiter, E. H., Serreze, D. V. & Coleman, D. L. (1987) *Science* **237**, 286–289.
7. Hattori, M., Buse, J. B., Jackson, R. A., Glimcher, L., Dorf, M. E., Minami, M., Makino, S., Moriwake, K., Kuzuya, H., Imura, H., Strauss, W. M., Seidman, J. G. & Eisenbarth, G. S. (1986) *Science* **231**, 733–735.
8. Wicker, L. S., Miller, B. J., Coker, L. Z., McNally, S. E., Scott, S., Mullen, Y. & Appel, M. C. (1987) *J. Exp. Med.* **165**, 1639–1654.
9. Hanafusa, T. & Tarui, S. (1990) *Curr. Top. Microbiol. Immunol.* **156**, 15–25.
10. Makino, S., Kunimoto, R., Muraoka, Y., Mizushima, Y., Katagiri, K. & Tochino, Y. (1980) *Exp. Anim.* **29**, 1–13.
11. Harada, M. & Makino, S. (1984) *Diabetologia* **27**, 604–606.
12. Acha-Orbea, H. & McDevitt, H. O. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2435–2439.
13. Ikegami, H., Eisenbarth, G. S. & Hattori, M. (1990) *J. Clin. Invest.* **85**, 18–24.
14. Todd, J. A., Bell, J. I. & McDevitt, H. O. (1987) *Nature (London)* **329**, 599–604.
15. Todd, J. A., Acha-Orbea, H., Bell, J. I., Chao, N., Fronek, Z., Jacob, C. O., McDermott, M., Sinha, A. A., Timmerman, L., Steinman, L. & McDevitt, H. O. (1988) *Science* **240**, 1003–1009.
16. Cornall, R. J., Prins, J. B., Todd, J. A., Pressey, A., DeLarato, N. H., Wicker, L. S. & Peterson, L. B. (1991) *Nature (London)* **353**, 262–265.
17. Garchon, H. J., Bedossa, P., Eloy, L. & Bach, J. F. (1991) *Nature (London)* **353**, 260–262.
18. Todd, J. A., Aitman, T. J., Cornall, R. J., Ghosh, S., Hall, J. R. S., Hearne, C. M., Knight, A. M., Love, J. M., McAleer, M. A., Prins, J. B., Rodrigues, N., Lathrop, M., Pressey, A., DeLarato, N. H., Peterson, L. B. & Wicker, L. S. (1991) *Nature (London)* **351**, 542–547.
19. Julier, C., de Gouyon, B., Georges, M., Guenet, J. L., Nakamura, Y., Avner, P. & Lathrop, G. M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4585–4589.
20. Avner, P., Amar, L., Dandolo, L. & Guenet, J. L. (1988) *Trends Genet.* **4**, 18–23.
21. Steinmeyer, K., Klocke, R., Ortland, C., Groonemeier, M., Jockusch, H., Grunder, S. & Jentsch, J. T. (1991) *Nature (London)* **354**, 304–308.
22. Oi, V. T., Jones, P. J., Goding, J. W., Herzenberg, L. A. & Herzenberg, L. A. (1978) *Curr. Top. Microbiol. Immunol.* **81**, 115–120.
23. Ozato, K., Mayer, N. & Sachs, D. H. (1980) *J. Immunol.* **124**, 533–540.
24. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed.
25. Love, J. M., Knight, A. M., McAleer, M. A. & Todd, J. A. (1990) *Nucleic Acids Res.* **18**, 4123–4130.
26. Hearne, C. M., McAleer, M. A., Love, J. M., Aitman, T. J., Cornall, R. J., Ghosh, S., Knight, A., Prins, J. B. & Todd, J. A. (1991) *Mammal. Genome* **1**, 273–282.
27. Aitman, T. J., Hearne, C. M., McAleer, M. A. & Todd, J. A. (1991) *Mammal. Genome* **1**, 206–210.
28. Cornall, R. J., Aitman, T. J., Hearne, C. M. & Todd, J. A. (1991) *Genomics* **10**, 874–881.
29. Dietrich, W., Katz, H., Lincoln, S. & Lander, E. (1992) *Genetics* **131**, 423–447.
30. Hilbert, P., Lindpaintner, K., Beckmann, J. S., Serikawa, T., Soubrier, F., Dubay, C., Cartwright, P., de Gouyon, B., Julier, C., Takahasi, S., Vincent, M., Ganten, D., Georges, M. & Lathrop, G. M. (1991) *Nature (London)* **353**, 521–529.
31. Lathrop, G. M. & Lalouel, J. M. (1984) *Am. J. Hum. Genet.* **36**, 460–465.
32. Donner, A. & Korval, J. (1980) *Biometrics* **36**, 19–25.
33. Morton, N. E. & MacLean, C. J. (1974) *Am. J. Hum. Genet.* **26**, 489–503.
34. Boney, G. E. (1984) *Am. J. Med. Genet.* **18**, 731–749.
35. Muller, U., Jongeneel, C. V., Nedospasov, S. A., Fischer-Lindahl, K. & Steinmetz, M. (1987) *Nature (London)* **325**, 265–267.
36. de Gouyon, B., Julier, C., Avner, P., Georges, M. & Lathrop, M. (1991) *Experimentia Suppl.* **58**, 85–94.
37. Lyons, M. & Searle, A. G. (1989) *Genetic Variants and Strains of Laboratory Mouse* (Oxford Univ. Press, Oxford), 2nd Ed.
38. Mock, B., Neumann, P. E., Eppig, J. & Huppi, K. (1991) *Mammal. Genome (Suppl.)* **1**, 241–268.
39. Wicker, L. S., Miller, B. J., Fischer, P. A., Pressey, A. & Peterson, L. B. (1989) *J. Immunol.* **142**, 781–784.