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Genetic Analysis of Autoimmune Sialadenitis in Nonobese Diabetic Mice: A Major Susceptibility Region on Chromosome 1

Olivier Boulard,* Guy Fluteau,* Laure Eloy,* Diane Damotte,† Pierre Bedossa,‡ and Henri-Jean Garchon2*†

The nonobese diabetic (NOD) mouse strain provides a good study model for Sjögren’s syndrome (SS). The genetic control of SS was investigated in this model using different matings, including a (NOD × C57BL/6 (B6))F2 cross, a (NOD × NZW)F2 cross, and ((NOD × B6) × NOD) backcross. Multiple and different loci were detected depending on parent strain combination and sex. Despite significant complexity, two main features were prominent. First, the middle region of chromosome 1 (chr.1) was detected in all crosses. Its effect was most visible in the (NOD × B6)F2 cross and dominated over that of other loci, including those mapping on chr.8, 9, 10, and 16; the effect of these minor loci was observed only in the absence of the NOD haplotype on chr.1. Most critically, the chr.1 region was sufficient to trigger an SS-like inflammatory infiltrate of salivary glands as shown by the study of a new C57BL/6 congenic strain carrying a restricted segment derived from NOD chr.1. Second, several chromosomal regions were previously associated with NOD autoimmune phenotypes, including Iddm (chr.1, 2, 3, 9, and 17, corresponding to Idd5, Idd13, Idd3, Idd2, and Idd1, respectively), accounting for the strong linkage previously reported between insulitis and sialitis, and autoantibody production (chr.10 and 16, corresponding to Bana2 and Bah2, respectively). Interestingly, only two loci were detected in the (NOD × NZW)F2 cross, on chr.1 in females and on chr.7 in males, probably because of the latent autoimmune predisposition of the NZW strain. Altogether these findings reflect the complexity and heterogeneity of human SS. The Journal of Immunology, 2002, 168: 4192–4201.

Sjögren’s syndrome (SS) is a chronic autoimmune disease characterized by lymphocytic infiltration and destruction of the salivary and lacrimal glands, causing oral and ocular dryness (1). It may develop primarily or be secondary and associated with other autoimmune manifestations, among which systemic lupus erythematosus and rheumatoid arthritis are the most frequent. The etiology of SS is presently unknown. It is likely to involve multiple factors, including immunologic, genetic, hormonal, and perhaps viral factors (1, 2).

When they exist, animal models are important for understanding a human disease. They are useful to clarify its causes, analyze its progression, and test novel treatments. Several mouse models for spontaneous SS are presently available, including the lupus-prone MRL-1pr and (NZB × NZW)F1, mice, and the NOD mouse strain (3–6). The latter strain is known primarily as a reference for insulin-dependent diabetes mellitus (Iddm). However, NOD mice also develop inflammatory infiltration of exocrine glands, notably including salivary and lacrimal glands (5). This model of sialadenitis has now been well characterized, and two features make it valuable for the study of human SS. First, the incidence of sialitis is gender-biased and is higher in females than in males (Refs. 7 and 8, and present work), as it is in human SS patients. Second, the inflammatory infiltration of the salivary glands is associated with a loss of secretory function (6). This infiltrate consists predominantly of CD4+ T cells that produce proinflammatory cytokines (9, 10) and also of some B lymphocytes that synthesize autoantibodies against components of the salivary glands (10, 11), notably against muscarinic and β-adrenergic receptors (12). Loss of secretory function could be mediated by these autoantibodies as shown by the study of NOD.IgMnull mice (13). Intrinsic anomalies of the salivary gland epithelium of NOD mice that do not depend on lymphoid cell infiltration have also been described previously (14–17).

The molecular mechanisms that underlie sialadenitis in NOD mice are unknown, but must be determined at least in part by genetic factors, as these mice have been inbred. A susceptibility locus was mapped on chromosome 1 (chr.1) in an initial genetic study from our laboratory (18) and was validated by the analysis of congenic mice (19). At the time of our initial study, few polymorphic genetic markers were available, and coverage of the genome was therefore partial. The genetic control of NOD sialitis, however, could be polygenic, as is the case for most autoimmune diseases and as was recently shown for sialitis in MRL-1pr mice (20). In the present study, we have completed the genome scan that we had initiated in search of sialitis loci in two F2 intercrosses, including a (NOD × B6)F2 cross and a (NOD × NZW)F2 cross. We have also used information from a ((NOD × B6) × NOD) backcross. In addition, for data analysis we have considered sialitis as a quantitative trait.

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Materials and Methods

Mice

NOD, C57BL/6 (B6), and NZW mice were bred and maintained under specific pathogen-free conditions in our facility at Institut National de la Santé et de la Recherche Médicale, Unité 25 (Hôpital Necker). We first generated (NOD × B6)F1, and (NOD × NZW)F1, hybrid mice and then the three crosses used in this study: (NOD × B6)F2, ((NOD × B6) × NOD)BC1, and (NOD × NZW)F2.

C57BL/6 mice congenic for the portion of chr.1 derived from the NOD strain between D1Mit18 and D1Mit26 (both markers included), covering ~30 cM, were obtained after 20 generations of iterative backcrossing with the NOD parent, followed by appropriate sister-brother matings.

Histopathology

Mice were sacrificed at the age of 10 mo. However, in the experiment shown in Fig. 8, mice were sacrificed at the age of 5 mo. Submandibulary salivary glands were removed and immersed in Bouin’s fixative. Three serial sections (5-µm thick) were stained with H&E and examined for mononuclear cell infiltration. Inflammatory foci were defined as an accumulation of at least 20 mononuclear cells. This threshold allowed us to account for the full range of infiltration occurring in progeny mice and was therefore appropriate for a quantitative trait analysis. The distribution of foci varied within the glands was heterogeneous. Therefore, whole sections rather than a selected area unit were examined, and data were expressed as the mean number of foci per section. The approximate area of the sections was 63.0 ± 5.8 mm^2 (mean ± SD of 10 glands).

Genotyping

Progeny mice were genotyped for polymorphic microsatellite markers identified in the Massachusetts Institute of Technology database (21) (http://www-genome.wi.mit.edu). For (NOD × NZW)F2 mice, markers had to be tested for polymorphism between NOD and NZW. However, alleles were not sized precisely, i.e., by sequencing gel electrophoresis. Qualitative information on these sizes compared among NZW, NOD, and B6 after agarose gel electrophoresis is available upon request. The following markers were used: D1Mit8, D1Mit11, D1Nds2, D1Mit16, D1Mit36, D2Mit2, D2Mit32, D2Nds1, D2Mit58, D2Mit17, D3Nds1, D3Mit10, Adh1, D3Mit9, D4Mit39, D4Mit35, Omy1(D4Mit12), D4Mit15, D4Mit28, D4Mit13, D5Mit48, D5Mit72, D5Nds2, D5Mit30, D6Mit223, Tcrb, D6Nds1, D6Mit4, D6Mit9, D6Mit10, Prp(D6Mit13), D6Mit14, D6Mit15, D7Mit20, Npyg(D7Nds5), D7Mit213, D7Mit53, D7Mit17, D7Mit242, D8N18, M2(D8Nds3), D8Mit40, D8Mit12, D8Mit88, D8Mit14, D9Mit42, Thy1(D9Nds3), Cypl2(D9Nds5), D9Nad1, D10Nad1, D10Mit2, D10Mit10, D10Mit14, B4, D11Nds1, D13Nds11, D13Nds15, D14Mit11, D14Mit41, D15Mit13, D15Mit10, D15Nds1, D15Mit29, Ly6, Hoxc15(D15Nds5), D16Mit55, D16Nds2, D16Mit178, D17Mit113, heat shock protein 70-1(D17Nds2), D17Mit36, D18Mit12, D18Mit17, D18Mit9, D18Mit4, D19Mit60, and D19Mit10.

The PCR was conducted following standard conditions as previously described (22), and PCR products were analyzed by electrophoresis on 5–6% agarose gels. At each marker, the NOD, B6, and NZW alleles were designated N, B, and W, respectively. The order of the markers and their distance to the centromere that were used in the text and tables were those indicated in the Mouse Genome Database (MGD) (23) (http://www.informatics.jax.org).

Statistical analysis and quantitative trait loci (QTL) mapping

The number of foci, or the sialitis score, was analyzed as a quantitative trait. The sample distribution was normal in ((NOD × B6) × NOD)BC1 females, and (NOD × NZW)F2 female mice. Otherwise, it was normalized by logarithmic transformation.

The association of each polymorphic marker with sialitis was tested with the Mapmaker/QTL program (24). Genetic maps used for QTL analysis were determined directly from the dataset with the Mapmaker/EXP 3.0 program (24) and were integrated into Mapmaker/QTL. There was no major difference between our linkage map and the MGD map. The mode of inheritance of each QTL was determined with the “try” command of Mapmaker/QTL. Data were also analyzed with QTL Cartographer (25, 26). The ZMapqtl module of this software implements composite interval mapping and, under model 6, considers background loci that include unlinked loci and linked loci located beyond a predefined window (10 cM in our case). A permutation test with 10,000 permutations was also performed to assess the probability of the data for each locus comparisonwise. Nonparametrics tests (Kruskal-Wallis or Mann-Whitney U) were systematically conducted to confirm the association and mode of inheritance at each locus. Interactions between loci were tested by ANOVA, and post hoc comparisons of means were performed with the honestly significant difference (HSD) test (Tukey). Calculations were made using STATISTICA for Windows software (Statsoft, Tulsa, OK).

Results

Phenotypic analysis of parental and F1 mice

Mice with at least one inflammatory focus in their salivary glands were considered affected. Analysis of sialadenitis in NOD parents indicated complete penetrance at 10 mo of age (Fig. 1) in both males (13 of 13 mice affected; mean score ± SD, 5.3 ± 2.6) and females (15 of 15 mice affected; score, 15.6 ± 5.4). Conversely, the infiltrate was minimal, although variable, among C57BL/6 male (8 of 12 mice affected; score, 1.6 ± 1.8) and female (6 of 10 mice affected; score, 1.7 ± 2.3) parents and NZW male (7 of 9 mice affected; score, 0.3 ± 0.5) and female (9 of 9 mice affected; score, 2.2 ± 1.6) parents. The (NOD × B6)F1 hybrids had a phenotype similar to that of their B6 parents (2 of 5 mice affected; score, 0.6 ± 0.9 for males; 3 of 5 mice affected; score, 1.2 ± 1.2 for females). The (NOD × NZW)F2 hybrids had an intermediate phenotype (5 of 5 mice affected; score, 2.4 ± 1.5 for males; 5 of 6 mice affected; score, 9 ± 2.5 for females). In the F2 generations also, the penetrance of sialitis was higher in females than in males. In fact, it was almost complete in females from the ((NOD × B6) × NOD) backcross and from the (NOD × NZW)F2 intercross (Fig. 1). The sialitis scores were also higher in the progeny of the backcross on the NOD parent than in the (NOD × B6)F2 intercross. This was consistent with the low expression of the trait in mice.
the (NOD × B6)F1 hybrids and suggested a role for recessive genes. In the (NOD × NZW)F2 female mice, the sialitis scores were higher than in (NOD × B6)F2 female mice, but they did not reach those of their NOD parents. Therefore, the NZW genetic background does not synergize with the NOD background to increase the severity of sialitis as it does with the NZB background to determine severe lupus manifestations (27).

Analysis of (NOD × B6)F2 cross

A genome-wide scan was performed on the (NOD × B6)F2 progeny with a total of 156 microsatellites markers. Their average spacing was 10 cM, the largest gap being 34 cM. Parametric analysis of the data with Mapmaker/QTL disclosed six chromosomal regions associated with sialitis (Fig. 2). Of these, only chr.1 was associated at a significant level (logarithm of odds (LOD) score, 4.8 at D1Mit11) according to proposed criteria (28). This major region extended over 50 cM, from D1Mit300 (MGD position 32.8 cM) to D1Mit104 (MGD position 79 cM), with several peaks having a LOD score >3 (Fig. 2). The strongest association was with the D1Mit11 marker (MGD position 58.7 cM) localized 1.1 cM centromeric to the Bcl2 gene, which was previously associated with sialitis (18). The best model to fit the inheritance of this susceptibility locus was recessive. As shown in Table I, mice homozygous for the NOD allele at this marker had a mean score of infiltration increased by 2-fold compared with mice carrying one or two B6 alleles.

The other five loci mapped to chr.8, 9, 10, 16, and 17 (Fig. 2). Evidence for their association was suggestive and was supported by nonparametric tests and analysis with the QTL Cartographer program using composite interval mapping with permutation tests (Table I). The NOD alleles were associated with susceptibility to sialitis at loci on chr.8, 9, and 17, but interestingly were protective at loci on chr.10 and 16. Modeling with the “try” command of the Mapmaker/QTL program indicated different modes of action for the susceptibility loci: additive on chr.8, recessive on chr.9, and dominant on chr.17. Conversely, on both chr.10 and 16 the NOD allele was dominantly protective. These adjustments were confirmed by nonparametric tests, showing smaller p values in each instance, which suggested better models (Table I).

Considering the marked sex bias of sialitis in the NOD parent and the (NOD × B6)F2 cross, the QTL analysis was also made separately in male and female mice. The H2 complex on chr.17 was the only region that was strictly dependent on sex; it was associated with sialitis only in females (QTL-LOD score, 3.9; nonparametric p = 6 × 10−4). The best model was dominant susceptibility for the NOD allele at this locus (QTL-LOD score, 3.9; p = 1 × 10−4).

**FIGURE 2.** Mapmaker/QTL linkage analysis of sialitis on indicated chromosomes in (NOD × B6)F2 mice. Analysis was model-free (heavy line) or used a dominant (dotted line), an additive (dashed line), or a recessive (thin line) model. Dominance and recessitivity were defined by reference to the NOD allele. The straight dashed line indicated the threshold for suggestive evidence of linkage (2.0) (28). The scales under QTL maps show distances in centimorgans.
is consistent with their dominant mode of inheritance. For loci on 
chr. 8 and 9 this may be explained by their pattern of interaction 
with the chr. 1 locus, as detailed below.

Conversely, we had detected no association on chr. 2 and 3 in the 
F2 cross despite a marked effect of these regions in the backcross 
progeny. Reconsidering the F2 cross data, we found no evidence 
for a role for chr. 3 in F2 females by single-locus analysis. However, 
combined analysis of the effects of chr. 1 and 3 by multiple 
regression disclosed an effect at the 
chr. 1 marker as detailed below.

Likewise, the three minor loci followed a dominant model. There-

model for the chr. 1 locus in the whole progeny was recessive, mice 
with NB and BB genotypes at the relevant markers were 
grouped. Both protective loci on chr. 10 and 16 interacted with the 
NOD allele. Interestingly, the protective effects of 
both loci on chr. 10 or 16. Interestingly, the protective effects of 
these two loci were additive, as best shown by testing a liability 
in an attempt to clarify the relationships between the multiple loci 
identified above, we examined their combined effects. We focused 
our analysis on the major locus on chr. 1 combined with the other 
minor loci. First, we tested the chr. 1 region vs the 3 dominant loci 
localized on chromosomes 10, 16, and 17 (Fig. 4). As the best 
model for the chr. 1 locus in the whole progeny was recessive, mice 
with NB and BB genotypes at the D1Mit11 marker were grouped. 
Likewise, the three minor loci followed a dominant model. Therefore, 
mice with NN and NB genotypes at the relevant markers were 
grouped. Both protective loci on chr. 10 and 16 interacted with the 
chr. 1 region; their protective effect was seen only in mice with the 
NB or BB genotypes at D1Mit11. The susceptibility determined by 
the chr. 1 locus was thus dominant over the protection afforded by 
either locus on chr. 10 or 16. Interestingly, the protective effects of 
these two loci were additive, as best shown by testing a liability 

Table I. Markers associated with sialitis in the (NOD × B6)F2 cross

<table>
<thead>
<tr>
<th>Chr.</th>
<th>Marker</th>
<th>Distancea (cM)</th>
<th>Genotype</th>
<th>No. of Mice</th>
<th>Sialitis Score (mean ± SD)</th>
<th>% Varianceb</th>
<th>QTL-LOD Scorec</th>
<th>QTL-LOD Scored</th>
<th>p′</th>
<th>p ′′</th>
<th>Model of Inheritancee</th>
<th>ρ ′</th>
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a Distance from the centromere given by the MGD.
b Percentage of variance explained by the locus (determined by Mapmaker/QTL).
c QTL-LOD score determined with Mapmaker/QTL.
d QTL-LOD score determined with QTL Cartographer by composite interval mapping (model 6). Genome-wide significance levels determined after 10,000 permutations: 4.04 for α = 0.05 and 6.15 for α = 0.01.
e Nominal p value determined with QTL Cartographer by composite interval mapping after 10,000 permutations.
f Nonparametric p value (Kruskall-Wallis test).
g Model of inheritance determined with the “try” command of Mapmaker/QTL.
h Modeled nonparametric p value (Mann-Whitney U test).

Analysis of the ((NOD × B6) × NOD) backcross

The aim of the study of the ((NOD × B6) × NOD) backcross was 
to examine the association of loci identified in the F2 cross and also 
of chromosomal regions previously associated with autoimmune 
diabetes in the NOD strain. Hence, only markers encompassing 
these regions were genotyped. The large size of the progeny, in-
cluding 203 females and 247 males, allowed us to perform a sex-
specific analysis.

In females, two loci, on chr. 1 and 3, showed linkage to sialitis 
(Fig. 3 and Table II). On chr. 1, the maximum LOD score was at 
the D1Mit5 marker (MGD position 32.8 cM), close to the peak 
mapped in F2 females. There seemed to be a second peak, more 
proximal, between markers D1Nds4 and D1Mit478 (Fig. 3), al-
though more extensive typing of this region is needed to rule out 
a computational artifact. The shift toward the centromere in back-
cross mice compared with F2 mice might reflect the presence of a 
second locus that would be recessive and more efficiently detected 
by a backcross. Expression of these loci might be also differential-
ly influenced by sex. Congenic mice should provide a congenic 
tool to dissect this region more finely. The locus on chr. 3 was 
centered on the Il2 gene, which is a candidate for the Id3 locus 
for autoimmune diabetes (29). For these two loci, the NOD allele 
conferring susceptibility to sialitis.

In males, only one locus, on chr. 2, showed suggestive linkage 
(Fig. 3 and Table II) with a maximum between the D2Mit378 and 
D2Mit14 markers. However, there was no defined peak, and the 
association followed a plateau across a large portion of chr. 2. Re-
markably, the NOD allele conferred protection against sialitis.

No association was found with loci that were identified on chr. 8, 
9, 10, 16, and 17 in the F2 cross. For loci on chr. 10, 16, and 17 this 
is consistent with their dominant mode of inheritance. For loci on 

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model (Fig. 5). In this model, mice with BB genotypes at both D10Mit257 and D16Mit195 markers were assigned a value of 0. Mice with a NN or NB genotype at one locus, exclusively of the other locus, were assigned a value of 1. Mice with an NN or NB genotype at both D10Mit257 and D16Mit195 were assigned a value of 2. By linear regression, the p value for the model was 10−10 and became 2 × 10−5 when the genotype at D1Mit11 was also taken into consideration. In contrast, there was no interaction

Table II. Markers associated with sialitis in the ((NOD × B6) × NOD) backcross

<table>
<thead>
<tr>
<th>Chr.</th>
<th>Marker</th>
<th>Distance (cM)</th>
<th>Genotype</th>
<th>No. of Mice</th>
<th>Sialitis Score (mean ± SD)</th>
<th>% Variance</th>
<th>QTL-LOD Score</th>
<th>p*</th>
</tr>
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<tbody>
<tr>
<td>Females</td>
<td>1</td>
<td>D1Mit5</td>
<td>32.8</td>
<td>NN</td>
<td>109</td>
<td>10.1 ± 4.2</td>
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<td>3</td>
<td>If2</td>
<td>19.2</td>
<td>NN</td>
<td>93</td>
<td>10.4 ± 4.7</td>
<td>8.0</td>
<td>3.62</td>
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<tr>
<td></td>
<td></td>
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<td></td>
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<td>Males</td>
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<td>D2Mit378</td>
<td>38.3</td>
<td>NN</td>
<td>118</td>
<td>1.2 ± 1.7</td>
<td>5.9</td>
<td>3.16</td>
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<td>NB</td>
<td>117</td>
<td>2.6 ± 3.7</td>
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</tr>
</tbody>
</table>

*Distance from the centromere given by the MGD.
*Percentage of variance explained by the locus (determined by Mapmaker/QTL).
*QTL-LOD score determined with Mapmaker/QTL.
*Nonparametric p value (Kruskall-Wallis test).

FIGURE 3. Mapmaker/QTL linkage analysis of sialitis on chr.1 and 3 in ((NOD × B6) × NOD) females and on chr.2 in males. The straight dashed line indicates the threshold for suggestive evidence of linkage (2.0) (28). The scales under QTL maps show distances in centimorgans.

FIGURE 4. Interactions between chr.1 and chr.10 (A), chr.16 (B), or chr.17 (C) in the (NOD × B6)F2 cross. Mice were grouped depending on their genotypes at D1Mit1 on chr.1 and at D10Mit257 on chr.10 (A), D16Mit195 on chr.16 (B), or heat shock protein 70 on chr.17 (C). The sialitis scores were normalized by logarithmic transformation. Means were calculated using all mice (affected and nonaffected) in each genotypic group. The number of affected mice over the total number of mice is indicated for each group. Comparison of the means was conducted with Tukey’s HSD test. Only significant p values are shown.
between chr.1 and 17 loci; their effects were simply additive (Fig. 4) even when females only were considered (data not shown).

Analysis of chr.1 vs the two remaining loci, on chr.8 and 9, disclosed a complex pattern of interaction. All combinations of possible genotypes were considered. As shown in Fig. 6, the predisposing effect of the NOD allele at chr.8 or 9 was significant only in mice with a BB genotype at D1Mit11. Therefore, although the chr.1 locus followed a recessive model of inheritance, there was a difference between the NB and BB genotypes regarding the effects of chr.8 or 9 loci. These findings, however, explain well the lack of effect of chr.8 and 9 in backcross mice. In contrast, there was no interaction between chr.1 and 3 loci in female ((NOD × B6) × NOD) backcross mice (Fig. 7). Their effects were additive.

FIGURE 5. Additive protection of chr.10 and 16 in the (NOD × B6)F2 cross. Mice were grouped depending on their genotypes at D10Mit257 on chr.10 and at D16Mit165 on chr.16. The sialitis scores were normalized by logarithmic transformation. Means were calculated using all mice (affected and nonaffected) in each genotypic group. The number of affected mice over the total number of mice is indicated for each group. Comparison of the means was conducted with Tukey’s HSD test. Only significant p values are shown.

FIGURE 6. Interactions between susceptibility loci on chr.1 and chr.8 or 9 in the (NOD × B6)F2 cross. Mice were grouped depending on their genotypes at D1Mit11 on chr.1 (indicated on the left) and at D8Mit190 on chr.8 (left panels) or at D9Nds1 on chr.9 (right panels). Sialitis scores were normalized by logarithmic transformation. Means were calculated using all mice (affected and nonaffected) in each genotypic group. The number of affected mice over the total number of mice is indicated for each group. Means were compared with Tukey’s HSD test. Only significant p values are shown.

FIGURE 7. Additive effect of chr.1 and 3 in ((NOD × B6) × NOD) BC1 females. Mice were grouped depending on their genotypes at D1Mit5 on chr.1 and at the Il2 gene on chr.3. Means were calculated using all mice (affected and nonaffected) in each genotypic group. The number of affected mice over the total number of mice is indicated for each group. Means were compared with Tukey’s HSD test.
Sialadenitis in C57BL/6 mice congenic for NOD chr.1

In a recent study, two congenic strains of C57BL/6 mice carrying large overlapping segments (44 and 47 cM, respectively) of chr.1 derived from the NOD strain (30) were found to display anomalies of their salivary glands, including inflammatory foci, increased cysteine protease activity, decreased amylase activity, and increased protein concentration in saliva (19). We derived a new C57BL/6 congenic strain carrying a restricted region from NOD chr.1. This region extended from D1Mit18 to D1Mit26, which are 32 cM apart, and corresponded to the overlap of the large segments harbored by the two congenic strains mentioned above. As shown in Fig. 8, at 20 wk of age these congenic mice displayed infiltration of their salivary glands by inflammatory foci of significant size (5 of 6 mice affected; mean ± SD number of foci, 3.6 ± 1.9), whereas six B6 mice of the same age showed no infiltration (p < 0.01 for comparison with congenics using Mann-Whitney U test). This observation indicated that the chr.1 region alone was sufficient to trigger an SS-like inflammatory infiltrate.

Analysis of the (NOD × NZW)F₂ cross

For the (NOD × NZW)F₂ intercross, a genome scan was performed with a more limited set of 87 markers that were polymorphic between the NOD and NZW strains (see detailed list in Materials and Methods). These markers were nevertheless evenly spaced, with a maximum gap of 40 cM. When considering the whole progeny (i.e., both females and males), suggestive evidence of linkage with sialitis was obtained for only two markers, both on chr.7 (Table III and Fig. 9). These two markers, D7Mit20 (centromeric) and D7Mit53 (telomeric), could correspond to two distinct loci, as they were separated by a marker, D7Nds5 (MGD position 23 cM, located in the Nγf gene), showing no linkage with sialitis. At both markers the NOD allele conferred dominant susceptibility.

Given the sex bias of sialitis, data were reanalyzed according to sex. In males the same two markers on chr.7 were detected again and flanked the nonassociated D7Nds5 locus, but the distal marker (D7Mit53) was now more significantly associated and showed significant linkage (Table III and Fig. 9). It is therefore possible that, at least in males, a major and a minor locus coexisted on chr.7.

In females, only one locus on chr.1 showed suggestive linkage (Table III). The maximum LOD score was at the D1Mit18 marker (MGD position 52 cM), which is included in the region associated with sialitis in (NOD×B6)F₂ females. The best model to fit inheritance at this locus was recessive. Of note, in each instance the NOD allele conferred susceptibility, and no protective locus was detected in this (NOD × NZW)F₂ cross. Interestingly, no association was found with the H2 region.

Discussion

Our study yields a complex picture of genetic control of the lymphocytic infiltration associated with SS in mice. Multiple and different loci were detected depending on sex, on the combination of strains used in the matings, and on the mating type itself. Moreover, although the NOD genome as a whole predisposes to SS, it does contain loci that are protective, at least in comparison with the C57BL/6 genome. Despite this complexity, the middle region of chr.1, which we had detected in our initial study (18), plays a major role in many respects. It was observed in all crosses studied to date, including two F₂ intercrosses, a (NOD × B6)F₂ cross and a (NOD × NZW)F₂ cross, and two backcrosses, a ((NOD × B6) × NOD) backcross and a (MRL-lpr × (MRL-lpr × C3H-lpr)) backcross (20). Its effect was most significant in the (NOD × B6)F₂ intercross, where a QTL-LOD score of 4.8 was reached. In this cross, it also was dominant over several other loci, including those mapping to chr.8, 9, 10 and 16, in that the effect of these minor loci was observed only in the absence of the NOD haplotype at the major locus on chr.1. Finally and most importantly, the study of C57BL/6 mice congenic for this region transferred from the NOD background has brought definitive confirmation of its implication in SS susceptibility (Ref. 19 and this study). The B6 mice congenic for chr.1 regions derived from the NOD strain that were studied by Brayer et al. (19) exhibited several features of autoimmune sialadenitis, including 1) morphological alterations with ductal hypertrophy, loss of acinar cell structures, and occasional lymphocytic infiltrates; and 2) biochemical modifications with increased protein concentration and decreased amylase activity of saliva, increased cysteine protease and metalloproteinase (MMP2 and MMP9) activities, and expression of the NOD-like 20-kDa isoform of parotid secretory protein in salivary gland extracts. The B6 congenic mice that we studied were derived independently and showed an overt mononuclear cell infiltration of their salivary glands. Taken together, these observations indicated that a single
regions must account for the strong linkage between SS and Iddm. The chr.2 region corresponds to SS were also involved in autoimmune phenotypes of NOD mice. Interestingly, the three loci that protected against NOD mice (18). Remarkably, this region of chr.1 was sufficient to trigger a spontaneous inflammatory infiltration of salivary glands.

Remarkably, this region of chr.1 was also associated with susceptibility to Iddm and is also known as the Idd5 locus (31). This was also the case for the three other SS loci identified in this work. These loci map to regions of chr.3, 9, and 17, which correspond to Idd3, Idd2, and Idd1, respectively (29, 32–34). These shared regions must account for the strong linkage between SS and Iddm in NOD mice (18). Interestingly, the three loci that protected against SS were also involved in autoimmune phenotypes of NOD mice. The chr.2 region corresponds to Idd13 that predisposes to Iddm in NOD mice (35). Chr.10 and 16 correspond to Bana2 (bacillus Calmette-Guérin-induced anti-nuclear Abs locus 2) and Bah2 (bacillus Calmette-Guérin-induced autoimmune hemolytic anemia locus 2), respectively, that also both protect NOD mice against the expression of defined autoantibodies (36). Whether the genes that control these various phenotypes in these common regions are the same is now open to investigation.

Whereas the study of mice congenic for Idd3 and Idd5 has confirmed the role of these loci in predisposition to SS (19), analysis of NOD.B10-H2 congenics led to the conclusion that the MHC played no role in murine SS (37). This discrepancy with our present data could be explained by the redundancy of SS predisposition loci in the NOD genome. Likewise, NOD mice that were single congenics for Idd5 or Idd3 showed no alteration of their salivary gland infiltration and secretory function compared with their NOD parent, whereas in double congenics the autoimmune exocrinopathy was significantly ameliorated, with an increase in amylase activity and flow rate of saliva, a decrease in cysteine protease and metalloproteinase activities, and a reduced number of inflammatory loci (19). Other Idd loci when studied in single congenics, including B6.NOD-Idd6, NOD.B6-Idd13 and NOD.B6-Idd9, did not modify the genetic background. Whether these loci, notably Idd13 in light of our results, would also interact with the chr.1 locus should be investigated. It remains that the H2-linked locus for murine SS does not have the critical importance it has in Iddm predisposition. It is nevertheless worthy of investigation because it may provide a model for understanding the contribution of HLA to predisposition to human SS (38, 39).

Few loci were identified in the (NOD × NZW)F2 cross. Additional loci could have been missed because of an incomplete genome coverage. Alternatively, few loci might be involved because the NZW strain also has an intrinsic predisposition to autoimmunity, notably including to Sle. At least three chromosomal regions predisposing to immune dysregulation are shared by NOD and NZW mice. Distal chr.1 is associated with defective expression of macrophage type II receptor for the Fc fragment of IgG (FcγRII), elevated serum levels of IgG (40), and anti-nuclear Abs in NOD mice (Bana3) (36), whereas it controls breakdown of B cell tolerance in NZW mice and corresponds to sle1 (41–43). Chr.4 harbors the Idd9 locus in NOD mice (44, 45) and the sle2 locus that

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Table III. Markers associated with sialitis in the (NOD × NZW)F2 cross

<table>
<thead>
<tr>
<th>Chr</th>
<th>Marker</th>
<th>Distance (cM)</th>
<th>Genotype</th>
<th>No. of Mice</th>
<th>Sialitis Score (mean ± SD)</th>
<th>% Variance</th>
<th>QTL-LOD Score</th>
<th>p*</th>
<th>Model of Inheritance</th>
<th>p'</th>
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<tbody>
<tr>
<td>7</td>
<td>D7Mit20</td>
<td>5.5</td>
<td>NN</td>
<td>52</td>
<td>4.2 ± 3.3</td>
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<td></td>
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<tr>
<td>7</td>
<td>D7Mit53</td>
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<td>NN</td>
<td>41</td>
<td>4.1 ± 2.9</td>
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<td>Dominant</td>
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<td>WW</td>
<td>50</td>
<td>2.9 ± 3.4</td>
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</tr>
</tbody>
</table>

Females

1  D1Mit8  52.0  NN  26  6.7 ± 2.8  10.9  2.14  0.010  Recessive  0.0025

Males

7  D7Mit53  51.8  NN  26  3.2 ± 2.5  19.5  4.72  9 × 10⁻⁵  Recessive  4.5 × 10⁻⁵

<table>
<thead>
<tr>
<th>Chr</th>
<th>Marker</th>
<th>Distance (cM)</th>
<th>Genotype</th>
<th>No. of Mice</th>
<th>Variance</th>
<th>QTL-LOD Score</th>
<th>p*</th>
<th>Model of Inheritance</th>
<th>p'</th>
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<tr>
<td>5</td>
<td>D5Mit20</td>
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<td>NW</td>
<td>28</td>
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<td>2.07</td>
<td>0.024</td>
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</table>

FIGURE 9. Mapmaker/QTL linkage analysis of sialitis on chr.7 in all (NOD × NZW)F2 mice and in males only. Analysis was model-free (heavy line) or used a dominant (dotted line), an additive (dashed line), or a recessive (thin line) model. Dominance and recessivity were defined by reference to the NOD allele. The straight dashed line indicated the threshold for suggestive evidence of linkage (2.0) (28). The scales under QTL maps show distances in centimorgans.
determines B cell hyperactivity in NZW mice (41, 46). Finally, the region of the H2 complex on chr.17 predisposes NOD mice to diabetes (32–34), sialitis (this work), and autoantibody expression (Bahl, Banal) (36) and is also the sles1 locus that suppresses the latent lupus-like autoimmunity of the NZW strain (47). The marked penetrance of sialitis in (NOD × NZW)F1 hybrids, notably in females, is also consistent with an involvement of few loci. Except for chr.1, different loci were detected in the NOD strain and in the MRL strain (20). This may be a result of the different combination of investigated strains, as MRL-lpr mice were mated with C3H-lpr mice. Yet this is also consistent with the difference in the SS phenotypes. No loss of secretory function of salivary glands was reported in MRL-lpr mice. Dendritic cells are also present in increased number at an early age in salivary glands of NOD mice compared with MRL-lpr mice (48). This diversity of phenotypes mimics the heterogeneity of SS in humans. Therefore, the comparative study of these two strains of mice and their genetic analysis using congenic lines should provide invaluable insight into the pathogenesis of SS.

Acknowledgments

We are most grateful to Isabelle Cisse for careful mouse breeding.

References


