High-Resolution Congenic Mapping of the Type 1 Diabetes Loci Idd10 and Idd18 in the NOD Mouse

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As many of the linked chromosome regions that predispose to type 1 diabetes in the NOD mouse have been dissected, it has become apparent that the initially observed effect is in fact attributable to several loci. One such cluster of loci on distal chromosome 3, originally described as Idd10, is now known to comprise three separate loci, Idd10, Idd17, and Idd18. Although these loci have a significant combined effect on diabetes development, their individual effects are barely detectable when diabetes is used as a read-out, which makes fine-mapping them by use of a conventional congenic approach impractical. In this study, we demonstrate that it is possible to map loci, with modest effects, to regions small enough for systematic gene identification by capitalizing on the fact that the combined loci provide more profound, measurable protection. We have mapped the Idd10 and Idd18 loci to 1.3- and 2.0-cM intervals, respectively, by holding the Idd3 allele constant. In addition, we have excluded Csfl and Nras as candidates for both loci. Diabetes 50:2633–2637, 2001

The nonobese diabetic (NOD) mouse is a spontaneous animal model of human type 1 diabetes. As is the case in the human disease, development of diabetes in the NOD mouse is under complex genetic control, requiring both major histocompatibility complex (MHC)-linked and -unlinked genes (1). At present, 17 insulin-dependent diabetes (Idd) loci have been chromosome-mapped in the NOD mouse in analyses involving the diabetes-resistant C57BL/6 (B6) or C57BL/10 strains (2–4), although none have been definitively identified. One approach that has been adopted for mapping Idd loci is the production of congenic mouse strains in which genetic material from a diabetes-resistant strain is introduced onto the NOD background (2,5–7). Although the genetic mapping of some loci has proved relatively straightforward, as with Idd3 on proximal mouse chromosome 3 (8–12), the situation has proved to be more complicated for other loci. Often, what was initially described as a single locus has broken down into multiple loci as the interval has been refined (3,4,7,8,13–15). Similar observations have been made for loci controlling other autoimmune diseases (16,17).

Idd10 was initially identified using congenic strains (8), demonstrating that the original linkage seen on chromosome 3 was due to at least two loci, Idd3 and Idd10. Subsequent analysis has shown that the original effect is in fact a result of the combination of three closely linked loci, Idd10, Idd17, and Idd18 (13,14). Whereas the Idd3 locus confers potent protection from diabetes development on its own (11), singularly, Idd10, Idd17, and Idd18 have very little protective effect (13,14). One consequence of this reduced level of protection is that further fine-mapping of these loci using a conventional congenic approach is impractical, as very large cohort sizes would be required to obtain significant differences in diabetes frequency with respect to the NOD parental strain.

Before the delineation of Idd10 and Idd18 into separate loci, it was observed that combined with Idd3, the Idd10/18 region produced almost complete protection from diabetes when the B6 allele of each locus was present in the homozygous state (13). Even when present in the heterozygous state, the loci interact to give significantly greater protection than either Idd3 or the Idd10/18 combination alone (13). After their resolution into separate loci, both Idd10 and Idd18 were individually combined with Idd3 (in a heterozygous state) to determine which was responsible for the interaction (14). Because the protective effect of Idd3 plus either locus alone was indistinguishable from that of Idd3 alone and significantly different from that observed when all three loci were present, both Idd10 and Idd18 were determined to be
required for the interaction (14). Given that both Idd10 and Idd18 need to be present to see any interaction with Idd3, we adopted the following strategy to more finely map both loci. The congenic strains NOD.B6 Idd3R450 (R450) (9) and NOD.B6 Idd10R2 (14) were intercrossed to produce a new strain, NOD.B6 Idd3 Idd10 Idd18 (NOD.Idd3/10/18) (Table 1), which carries two distinct segments of B6-derived DNA on chromosome 3. One segment, between the microsatellite markers D3Nds55 and D3Nds76, encompasses the Idd3 interval. The other segment, between D3Mit157 and D3Mit124, contains both the Idd10 and Idd18 intervals. The NOD.Idd3/10/18 strain was backcrossed to R450, and the resulting F1 progeny were intercrossed. The F2 progeny of this cross were genotyped with microsatellite markers across the D3Mit157 and D3Mit124 interval to identify mice carrying recombinant events within either Idd10 or Idd18. Informative recombinant events were fixed to homozygosity, and diabetes frequencies were determined for each of the new strains (Fig. 1A and B). Because mice that carry a B6-derived allele of Idd3, with either Idd10 or Idd18 alone but not combined, have a cumulative diabetes frequency similar to that of Idd3 alone, strains carrying recombinant events that lead to the loss of either locus should have diabetes frequencies significantly greater than that of the parental NOD.Idd3/10/18 strain.

Two sets of new subcongenic strains were developed (Table 1). The first set consists of three strains, NOD.B6 Idd3 Idd18R393 (R393), NOD.B6 Idd3 Idd10 Idd18R323 (R323), and NOD.B6 Idd3 Idd10 Idd18R53 (R53), which carry recombinant events within the previously defined 2.1-cM Idd10 interval between the microsatellite markers D3Mit157 and D3Nds35 (Table 1). Female mice of the R53 congenic strain have a diabetes frequency of 8.9% (9 of 101) at 7 months of age. By Kaplan-Meier analysis, diabetes progression in this strain is significantly different from that of the R450 strain (8.9 vs. 18.9%, P = 0.03) (Table 2 and Fig. 1A) but similar to that of the NOD.Idd3/10/18 strain (8.9 vs. 9.4%, P = 0.908) (Table 2 and Fig. 1A), suggesting that the R53 congenic strain retains both Idd10 and Idd18. This would place Idd10 distal of the microsatellite marker D3Mit213 (Table 1). The R93 strain has a female diabetes frequency of 20.3% (14 of 69) (Fig. 1A and Table 2) at 7 months of age. Although diabetes progression is identical to that of the R450 strain (P = 0.715) (Table 2), it is significantly different from that of the NOD.Idd3/10/18 strain (P = 0.027) (Table 2). This suggests that the R93 strain has lost Idd10 and places the distal boundary of Idd10 in the 0.92-cM interval between D3Mit189 and D3Nds35 (Table 1), confirming our previous mapping data (14). The R323 congenic strain has a female diabetes frequency of 9.4% (5 of 53) (Fig. 1A and Table 2) at 7 months of age. Although this is not significantly different from either the R450 or NOD.Idd3/10/18 strains (P = 0.103 and P = 0.991, respectively) (Table 2), there is a trend toward it being more similar to the NOD.Idd3/10/18 strain than the R450 strain (Fig. 1A). If the R323 data are combined with that of the strains that definitively retain both Idd10 and Idd18 (R53 and NOD.Idd3/10/18) and compared with the strains that don’t contain Idd10 (R450 and R93), the two groups are significantly different (P = 0.001). Taken together, these data suggest that the R323 strain has retained both the Idd10 and Idd18 genes. This would place the proximal boundary of Idd10 in the 0.35-cM interval between D3Mit10 and D3Mit12 (Table 1), mapping the locus to the 1.3-cM interval between, but not including, the markers D3Mit10 and D3Nds35 (Table 1).

The second set of new congenic strains, NOD.B6 Idd3 Idd10R1 (R1), NOD.B6 Idd3 Idd10R47 (R47), and NOD.B6 Idd3 Idd10 Idd18R135 (R135), carries recombinant events between the markers D3Mit103 and D3Mit109, within the previously defined 5.1-cM Idd18 interval (Table 1). The female diabetes frequencies of the R1, R47, and R135 strains were 17.0% (8 of 47), 17.7% (18 of 102), and 12.8% (11 of 86), respectively, at 7 months of age (Fig. 1B and Table 2). Although none of the individual strains are statistically different from either the R450 or NOD.Idd3/10/18 strains, a clear trend was apparent (Fig. 1B). The strains appear to fall into two groups, with the R135 strain being similar to the NOD.Idd3/10/18 strain, whereas the R1 and R47 strains are similar to the R450 strain (Fig. 1B). By Kaplan-Meier analysis, the combined data for the two groups of strains are significantly different (NOD.Idd3/10/18 and R135 vs. R1, R47, and R450, P = 0.0229), suggesting that the Idd18 locus has been lost from the R1 and R47 strains but retained by the R135 strain. This places Idd18 in the 2.0-cM interval between, but not including, the markers D3Nds95 and D3Mit106 (Table 1).

Congenic strains are a powerful tool for excluding, as well as including, potential candidate genes. In previous studies, we have used congenic strains to exclude a number of candidate genes for Idd10 and Idd18, including Fgpr1 and Cd2 (13,14). Another potential candidate gene that has been mapped close to the Idd10/18 region is Csf1, which encodes macrophage colony–stimulating factor 1 (Mouse Genome Database, http://www.informatics.jax.org, December 2000). To facilitate its analysis, a Csf1-positive clone, mP28i5, was isolated from a mouse P1 genomic library and sequenced. The genomic structure of the Csf1 gene was determined by aligning the clone sequence with the sequences of two alternatively spliced Csf1 mRNAs. The Csf1 gene is comprised of 10 exons (Table 3), with the Csf1 protein encoded by exons 1–8. The alternatively spliced transcripts are generated via the splicing of either exons 9 or 10 to exon 8. Each exon, plus ~1 kb of the putative promoter region, was sequenced from NOD and B6 to identify variants that might lead to changes in the amino acid sequence or expression of the gene. No sequence variants were observed between the two strains (data not shown). At the same time, a microsatellite marker for Csf1, D3Nds93, was developed and used to position Csf1, with respect to Idd10 and Idd18, by genotyping the 149 F2 progeny of a cross between the R450 and NOD.B6 Idd10R2 strains. Csf1 colocalizes with D3Mit103 0.34 cM (1 recombinant in 298 meioses tested) proximal to D3Nds95 within the congenic segment of the R1 strain (Table 1). This places Csf1 between Idd10 and Idd18 and excludes it as a candidate for either locus.

Another potential candidate gene mapping close to the Idd10/18 region is Nras, which encodes the neuroblastoma ras oncogene. The 5’ sequence of Nras was determined for both the NOD and B6 strains (data not shown). Although no variants were identified within the coding region of the gene scanned, a 3-bp deletion was found in...
Table 1 — Congenic strains defining the Idd10 and Idd18 loci

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B6 or NOD indicates B6.PL Thy1" or NOD homozygosity at locus. NT indicates not typed. The boxed marker loci indicate the positions of Idd3, Idd17, Idd10, and Idd18, as defined in previous studies (11,13,14). The shaded marker loci indicate the positions of the newly defined Idd10 and Idd18 intervals. aMarker order and distances taken from Podolin et al. (14); bintermarker distance determined from Podolin et al. (13).
the 5′ untranslated region of the NOD strain at position −25 (numbering relative to the first nucleotide of the start codon). To more precisely map \textit{Nras} relative to \textit{Idd10} and \textit{Idd18}, each of the new congenic strains was genotyped for the \textit{NOD} strain at position 5′/H11032 (\textit{Idd10}), 5′/H11002 (\textit{NOR}), and 5′/H11549 (\textit{Idd3/10/18}). Each of the new congenic strains was genotyped for \textit{Nras} codon). To more precisely map

\begin{table}
\centering
\caption{Diabetes frequencies in female congenic mice}
\begin{tabular}{llll}
\hline
Congenic strain & Diabetes frequency & \textit{P} vs. R450 & \textit{P} vs. NOD.\textit{Idd3/10/18} \\
\hline
R450 & 18.9% (17/90) & -- & 0.049 \\
NOD.\textit{Idd3/10/18} & 9.4% (10/106) & 0.049 & -- \\
R53 & 20.3% (14/69) & 0.715 & 0.027 \\
R323 & 8.9% (9/101) & 0.03 & 0.908 \\
R1 & 9.4% (5/53) & 0.103 & 0.991 \\
R47 & 17.0% (8/47) & 0.793 & 0.145 \\
R135 & 17.7% (18/102) & 0.741 & 0.078 \\
\end{tabular}
\end{table}

(data not shown), placing it between \textit{Idd10} and \textit{Idd18} and excluding it as a candidate for either locus.

As the fine-mapping of \textit{Idd} loci has progressed, it has become apparent that many of the initial effects mapped using linkage analysis are the result of the combination of multiple loci, each of which has a modest protective effect. One notable exception to this is the \textit{Idd3} locus (11). One consequence is that the fine-mapping of these loci to the resolution required for physical mapping will be impractical using a conventional congenic approach. As demonstrated here, by combining loci and keeping one constant while generating new recombinants within the other, even loci with relatively modest effects can be mapped to a resolution suitable for systematic gene identification. This approach will be directly applicable to mapping other loci, with relatively small individual effects, such as \textit{Idd5.1} and \textit{Idd5.2} on chromosome 1 (4). The BAC cloning and sequencing of \textit{NOD} and B6 DNA from the \textit{Idd10} interval, defined here, is now underway.

\begin{table}
\centering
\caption{The intron/exon structure of the mouse \textit{Csf1} gene}
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Exon & Nucleotide* & 5′ splice donor & 3′ splice acceptor† & Intron size (bp) \\
\hline
Exon 1 & 1–213 & TGAAA & CTTCGGtaag & 3,285 \\
Exon 2 & 214–336 & tataAGATG & AGTGGtaag & 1,121 \\
Exon 3 & 337–390 & cacatCTCGA & AGCTGgtaag & >1,127 \\
Exon 4 & 400–570 & tccacATGAA & ACAAGgtaag & 3,273 \\
Exon 5 & 571–718 & tccacGCTCG & CCGAGGgtaag & 1,140 \\
Exon 6 & 719–734 & ttcagATGGA & GAGGGAgaag & 993 \\
Exon 7 & 735–787 & ttcagAGCCA & GACAGGgtaag & 322 \\
Exon 8 & 788–846 & ttcaAGCCTCC & CATGGAgaag & 879 \\
Exon 9 & 847–2,000 & ttcaGACCC & GTTCT & 2,459 \\
Exon 10 & 1,847–3,937 & ttcaGCTGGG & GCTGC & \\
\end{tabular}
\end{table}

\textbf{RESEARCH DESIGN AND METHODS}

\textbf{Mice.} NOD/Mrtac mice were purchased from Taconic Farms (Germantown, NY). The derivation of the congenic strains R450 (N13F5-6) and NOD.B6 \textit{Idd10R2} (NISP9) has been previously described (9,14). During their development, progenitor mice for both strains were tested for the presence of non–NOD-derived DNA at microsatellite markers spread across the genome and were found to be NOD at all loci (10). The congenic strains NOD.B6 \textit{Idd3 Idd10 \textit{Idd18}} (N10F6-7), NOD.B6 \textit{Idd3 Idd18R3} (N12F2-3), R323 (N12F3), NOD.B6 \textit{Idd3 Idd10 Idd18R3} (N12F2-3), NOD.B6 \textit{Idd3 Idd10 \textit{Idd18R35}} (N12F2-3), NOD.B6 \textit{Idd3 Idd10 R1F1} (N12F2-3), and NOD.B6 \textit{Idd3 R1F1} (N12F2-3) were generated essentially as previously described (13). The congenic strains R450, NOD.B6 \textit{Idd10R2}, and R323 are available from the Taconic Farms Emerging Models Program (Lines 1098, 1101, and 1538, respectively). Grants to enable the public distribution of the lines have been given by the Merck Genome Research Institute, the Juvenile Diabetes Foundation International, the Wellcome Trust, and the National Institute of Allergy and Infectious Diseases. All animals were housed under specific pathogen-free conditions.

\textbf{Assessment of diabetes.} Mice were tested for diabetes as previously described (13). All diabetes frequencies were contemporaneously determined.

\textbf{Statistical analysis.} The Kaplan-Meier Wilcoxon test was used to compare the diabetes frequency curves of the NOD congenic strains. \textit{P} < 0.05 was considered statistically significant.

\textbf{Genotyping.} Microsatellite genotyping was performed as previously described (9). Primer sequences for \textit{D3NdsXX} markers can be found on our website (http://diesel.cimr.cam.ac.uk/todd).

\textbf{Determination of the \textit{Csf1} gene exon structure.} The ICRF mouse P1 genomic library was screened, and a clone positive for \textit{Csf1} was identified as previously described (18). The clone was shotgun sequenced as previously described (3), and the intron/exon structure of the \textit{Csf1} gene was determined.
by aligning the genomic sequence to two alternatively spliced Csf1 mRNA sequences in Genbank (accession nos. M21149 and M21952). Individual exons were amplified using nested polymerase chain reaction (PCR) primer sets, and the PCR products were directly sequenced as previously described (18).

**Nras sequencing.** Nested PCR primer pairs were designed to the available *Nras* genomic sequence (accession no. L19607). PCR products were sequenced directly as previously described (18).

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**REFERENCES**