Colocalization of Mouse Autoimmune Diabetes Loci
Idd21.1 and Idd21.2 With IDDM6 (Human) and Iddm3 (Rat)

Jade E. Hollis-Moffatt,1 Sarah M. Hook,2 and Tony R. Merriman1

Comparative mapping between the human and rodent genomes is one approach for positional cloning of complex disease loci. The human type 1 diabetes susceptibility locus IDDM6 has orthology with distal rodent chromosome 18, to which Iddm3 has been mapped in rat. Previously, we mapped Idd21 to mouse chromosome 18. Here, the primary aim was to determine whether Idd21 mapped to distal mouse chromosome 18. We constructed novel congenic strains from the consomic NOD-Chr18ABH strain and mapped two loci (Idd21.1 and Idd21.2) to the distal 29.3-Mb portion of mouse chromosome 18, orthologous to IDDM6 (human) and Iddm3 (rat). Idd21.3 was mapped to proximal mouse chromosome 18 (0–21.9 Mb). Although Idd21.1 did not influence β-islet inflammation, splenocytes from prediabetic Idd21.1-congenic mice were less efficient at transferring diabetes to immunodeficient NOD-scid mice. This suggests that Idd21.1 may act by reducing the pathogenicity of islet-infiltrating immune cells. For the first time, the presence of a non–major histocompatibility complex autoimmune diabetes locus colocalizing in three species has been demonstrated; IDDM6 (human), Iddm3 (rat), and now Idd21.1–21.2 in mouse. Further genetic localization of Idd21.1 and Idd21.2 could expedite characterization of the human IDDM6 region.

Diabetes 54:2820–2825, 2005

The nonobese diabetic (NOD) mouse has many similarities with humans in the pathology of autoimmune diabetes. This is reflected in overlap in genetic susceptibility: serine at position 57 of MHC-DQB1 in human correlates with diabetes susceptibility (1), and the NOD mouse DQ ortholog (I-A) has a unique β-chain with serine at position 57 (2); specific isoforms of CTLA4 have been strongly implicated in autoimmune diabetes etiology in both human and mouse (3). Human IDDM6 on chromosome 18q12–q21 contains extensive orthology with distal rodent chromosome 18, which has previously been implicated in susceptibility to autoimmune phenotypes (4). Furthermore, Iddm3 has been mapped by congenic analysis to distal chromosome 18 in the biobreeding rat (5). We previously used the consomic NOD-Chr18ABH strain to demonstrate Idd21 on mouse chromosome 18 (6). Here, the primary aim was to test whether Idd21 mapped to the IDDM6/Iddm3-orthologous distal portion of mouse chromosome 18. If so, then comparative mapping could prove a useful strategy in IDDM6 characterization, as was the case with CTLA4 (3).

The experimental strategy was to create a series of congenic strains containing, from the distal end, increased amounts of chromosome 18 ABH-derived DNA (Fig. 1). Any difference in diabetes incidence between congenic strains could be credited to the ABH-derived DNA that distinguished the strains. Ensembl (build m33; available at www.ensembl.org/Mus_musculus) was used to develop the physical map used to establish the congenic lines.

Diabetes incidence in the congenic strain that contained ABH-derived DNA only, within the distal portion of chromosome 18 [NOD.ABH-(D18Mit18-D18Mit4) (NE8), 52% diabetes incidence at 30 weeks] was significantly reduced compared with NOD (Fig. 2A, Table 1; 85%, P < 0.0001). The diabetes incidence of NOD.ABH-(D18Mit18-D18Mit4) was confirmed by reconstituting this strain at the NE11 generation [Fig. 1A; 50%, P = 0.61 comparing NOD.ABH-(D18Mit18-D18Mit4) NE8 and NE11 diabetes incidences]. This evidence supports the mapping of Idd21.1 (ABH resistance allele) to a maximal interval consisting of the distal 18.1 Mb of chromosome 18.

Diabetes incidence in NOD.ABH-(D18Mit18-D18Mit4) (NE8) was significantly reduced compared with each of three distinct strains containing up to 30.9 Mb of additional ABH-derived DNA in the distal half of the chromosome [Fig. 1, Fig. 2A, and Table 1; NOD.ABH-(D18Mit207-D18Mit4), NOD.ABH-(D18Mit124-D18Mit4), and NOD.ABH-(D18Mit235-D18Mit4); all P ≤ 0.0002]. These data support the mapping of Idd21.2 to a maximal interval of 13.3 Mb (61.7–75.0 Mb). There is a significant difference in diabetes incidence between NOD.ABH-(D18Mit18-D18Mit4) and NOD.ABH-(D18Mit207-D18Mit4) (P < 0.0001), whereas the diabetes incidences between NOD.ABH-(D18Mit124-D18Mit4), NOD.ABH-(D18Mit124-D18Mit4), and NOD.ABH-(D18Mit235-D18Mit4) were

From the 1Department of Biochemistry, University of Otago, Dunedin, New Zealand; and the 2School of Pharmacy, University of Otago, Dunedin, New Zealand.

Address correspondence and reprint requests to Dr. Tony Merriman, University of Otago, Department of Biochemistry, Box 56, Dunedin, New Zealand. E-mail: tony.merriman@stonebow.otago.ac.nz.

Received for publication 28 September 2004 and accepted in revised form 21 June 2005.

© 2005 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
similar ($P > 0.66$). Thus, in the distal region of mouse chromosome 18, orthologous to part of human IDDM6 and rat Iddm3, an additional locus was mapped (Idd21.2; ABH susceptibility allele).

Diabetes incidences in the congenic strains containing increasing amounts of chromosome 18 ABH-derived DNA in the middle portion (21.9–44.1 Mb) were compared. The NOD.ABH-(D18Mit235-D18Mit4) strain had a significantly increased diabetes incidence compared with NOD.ABH-(D18Mit37-D18Mit4) (66 vs. 51%, $P = 0.003$) but not when compared with NOD.ABH-(D18Mit111-D18Mit4) (59%, $P = 0.11$). It is possible that an Idd locus is contained in the 21.9- to 44.1-Mb interval. However, to confirm this a chromosome 18 congenic strain containing ABH-derived DNA only in the D18Mit111-D18Mit235 interval would need to be studied. As this region is not orthologous to any human type 1 diabetes locus, it was not pursued.

The consomic NOD-Chr18ABH strain (36%) had a reduced diabetes incidence compared with NOD.ABH-(D18Mit64-D18Mit239) (59%, $P < 0.0001$). This strain had a significantly reduced diabetes incidence compared with NOD (65 vs. 85%, $P < 0.0001$).

The possibility that the differences in diabetes incidence between the novel chromosome 18 congenic strains is due to undetected regions of extra-chromosome 18 ABH-derived DNA, rather than to Idd loci mapping within the regions of chromosome 18 ABH-derived DNA differential-

FIG. 1. Novel NOD.ABH-(Chr18) congenic strains. The eight framework markers are in bold. Notable candidate genes and physical positions are: Il17b (62.2 Mb), Trag (64.2 Mb), Tcf7 (70.0 Mb), Dec (71.8–72.9 Mb), Smad4 (74.2 Mb), Smad7 (75.9 Mb), and Smad3 (76.8 Mb). The maximal Idd21.1–21.3 intervals are boxed (the maximal interval for both Idd21.1 and Idd21.2 overlaps [shaded area]). The bold dashed box indicates the orthology to rat Iddm3.
FIG. 2. A: Cumulative diabetes frequency (CDF) in cohorts of NOD/MrkTac (●), NOD.ABH-(D18Mit8-D18Mit4) (NE8) (○), NOD.ABH-(D18Mit8-D18Mit4) (NE11) (– – –), NOD.ABH-(D18Mit207-D18Mit4) (■), NOD.ABH-(D18Mit124-D18Mit4) (□), NOD.ABH-(D18Mit5-D18Mit4) (×), and NOD-Chr18<sup>3<sup<</sup> (NE8) (▲). B: CDF in cohorts of NOD/MrkTac (●), NOD.ABH-(D18Mit235-D18Mit4) (×), NOD.ABH-(D18Mit37-D18Mit4) (■), NOD.ABH-(D18Mit111-D18Mit4) (○), NOD-Chr18<sup>3<sup< (NE8) (▲), NOD-Chr18<sup>3<sup< (NE5) (■), and NOD.ABH-(D18Mit164-D18Mit239) (○). C: Transfer of diabetes into NOD-scid by splenocytes from NOD (n = 79 recipients; ■) and NOD.ABH-(D18Mit8-D18Mit4) (n = 72 recipients; ○).
ing the strains, needs to be considered. Without performing a thorough genome scan, it is not possible to definitively exclude an effect of extra–chromosome 18 ABH-derived DNA. However, the close similarity in diabetes incidence between the NE8 and NE11 NOD.ABH-(D18Mit8-D18Mit4) lines and the NE5 and NE8 NOD-Chr18<sup>ABH</sup> consomic lines (Fig. 2A and B; <i>P</i> = 0.61 and 0.48, respectively) provides no evidence for the existence of sufficient extra–chromosome 18 ABH-derived DNA segments influencing diabetes incidence in the strains tested here. These data, and the consistency of diabetes incidence in our NOD cohort over time (RESEARCH DESIGN AND METHODS), also demonstrate the reproducibility of diabetes incidence in our facility in assessments done at different times.

Focusing on <i>Idd21.1</i>, which contains extensive orthology with human IDDM6, the influence of this locus on insulinis was investigated (Fig. 3). Levels of insulinis were not significantly different between NOD and NOD.ABH-(D18Mit8-D18Mit4) (<i>P</i> = 0.39), indicating that the reduced diabetes incidence of NOD.ABH-(D18Mit8-D18Mit4) is due either to reduced pathogenicity of islet-infiltrating immune cells and/or intrinsic resistance of β-cells to cytotoxic effects of the infiltrating immune cells. The ability of NOD.ABH-(D18Mit8-D18Mit4) (NES) splenocytes to transfer diabetes was then investigated (Fig. 2C). They were less efficient at transferring diabetes to NOD-scid than NOD splenocytes (<i>P</i> < 0.0001), suggesting that <i>Idd21.1</i> may act by reducing the pathogenicity of islet-infiltrating immune cells. However, 87% of recipient mice developed diabetes when injected with the splenocytes from NOD.ABH-(D18Mit8-D18Mit4) (<i>Idd21.1</i>) compared with 52% of spontaneous diabetes in NOD.ABH-(D18Mit8-D18Mit4). Thus, <i>Idd21.1</i> splenocytes become more diabetogenic in NOD-scid. Possible explanations for this could be that <i>Idd21.1</i> splenocytes are able to develop into more effective β-cell cytotoxic cells and/or cytotoxic <i>Idd21.1</i> splenocytes could be under less effective negative regulation (owing to interaction with the recipient NOD-scid innate immune system and/or exposure to NOD-scid β-islets). Similar adoptive transfer data have been reported for <i>Idd4</i> (7).

Each of <i>Idd21.1</i>–21.3 contain orthology to a region of the human genome implicated in type 1 diabetes. Both <i>Idd21.1</i> and <i>Idd21.2</i> have extensive regions of orthology with IDDM6 (chromosome 18q12-q21; 4). The presence of two autoimmune diabetes loci in the IDDM6-orthologous region raises the possibility that the IDDM6 linkage, in humans, may be due to more than one locus. Fine mapping the loci underlying <i>Idd21.1</i> and <i>Idd21.2</i> will enable this possibility to be explored further. The proximal region of <i>Idd21.3</i> contains orthology to IDDM10 (chromosome 10p11-q11; 8). Further refinement of <i>Idd21.3</i> using congeneric mapping will determine whether <i>Idd21.3</i> and IDDM10 colocalize.

Candidate genes of particular interest within the IDDM6-orthologous interval are Smad2 and Smad7 (<i>Idd21.1</i>), Smad4 (either <i>Idd21.1</i> or <i>Idd21.2</i>), Dcc, and Tgfβ4 (<i>Idd21.2</i>). The Smad (similar to mothers against decapentaplegic) proteins mediate signaling of Tgfβ, an immunosuppressive cytokine strongly implicated in autoimmunity (9). Mapping in <i>Idd21.2</i> are the Dcc (deleted in colorectal

---

**Table 1**

<table>
<thead>
<tr>
<th>Gene</th>
<th>NOD</th>
<th>NOD-Chr18&lt;sup&gt;ABH&lt;/sup&gt;</th>
<th>NOD.ABH-(D18Mit111-D18Mit4)</th>
<th>NOD.ABH-(D18Mit35-D18Mit4)</th>
<th>NOD.ABH-(D18Mit235-D18Mit4)</th>
<th>NOD.ABH-(D18Mit124-D18Mit4)</th>
<th>NOD.ABH-(D18Mit207-D18Mit4)</th>
<th>NOD.ABH-(D18Mit8-D18Mit4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>n</td>
<td>178</td>
<td>239</td>
<td>188</td>
<td>171</td>
<td>179</td>
<td>179</td>
<td>171</td>
<td>168</td>
</tr>
</tbody>
</table>

---

**METHODS**

In conclusion, the nearly complete absence of significant differences in diabetes incidence between NOD and NOD.ABH-(D18Mit8-D18Mit4) and NOD.ABH-(D18Mit111-D18Mit4) lines indicates that diabetes in NOD.ABH-(D18Mit8-D18Mit4) is not due to a lack of diabetes-susceptible genetic material in this region. Instead, the close similarity in diabetes incidence between the NE8 and NE11 NOD.ABH-(D18Mit8-D18Mit4) lines and the NE5 and NE8 NOD-Chr18<sup>ABH</sup> consomic lines (Fig. 2A and B; <i>P</i> = 0.61 and 0.48, respectively) provides no evidence for the existence of sufficient extra–chromosome 18 ABH-derived DNA segments influencing diabetes incidence in the strains tested here. These data, and the consistency of diabetes incidence in our NOD cohort over time (RESEARCH DESIGN AND METHODS), also demonstrate the reproducibility of diabetes incidence in our facility in assessments done at different times.

---

**Figure 3**

**Figure 3** shows the comparison of diabetes incidence between NOD and NOD.ABH-(D18Mit8-D18Mit4) lines and the NE5 and NE8 NOD-Chr18<sup>ABH</sup> consomic lines (Fig. 2A and B; <i>P</i> = 0.61 and 0.48, respectively) provides no evidence for the existence of sufficient extra–chromosome 18 ABH-derived DNA segments influencing diabetes incidence in the strains tested here. These data, and the consistency of diabetes incidence in our NOD cohort over time (RESEARCH DESIGN AND METHODS), also demonstrate the reproducibility of diabetes incidence in our facility in assessments done at different times.

---

**Figure 2C**

**Figure 2C** shows the comparison of diabetes incidence between NOD and NOD.ABH-(D18Mit8-D18Mit4) lines and the NE5 and NE8 NOD-Chr18<sup>ABH</sup> consomic lines (Fig. 2A and B; <i>P</i> = 0.61 and 0.48, respectively) provides no evidence for the existence of sufficient extra–chromosome 18 ABH-derived DNA segments influencing diabetes incidence in the strains tested here. These data, and the consistency of diabetes incidence in our NOD cohort over time (RESEARCH DESIGN AND METHODS), also demonstrate the reproducibility of diabetes incidence in our facility in assessments done at different times.
carcinoma) gene, which encodes a protein which mediates caspase-3–dependent apoptotic signals (10) and Tcf4 (immunoglobulin transcription factor 2). In humans, DCC has previously been associated with autoimmune diabetes (4).

Meta-analysis of published genome-wide linkage scans provided evidence of the potential importance of distal rodent chromosome 18 in susceptibility to rodent autoimmunity, with linkages reported to induced arthritis, experimental allergic encephalomyelitis, systemic lupus erythematosus, and diabetes (4 and references therein). This region has now been shown by congenic analysis to be important in rodent autoimmune diabetes (Idd21.1 and Idd21.2 in mouse [here] and Iddm3 in rat [5]). Further congeneric data emphasizing the relevance of this region in rodent autoimmune diabetes comes from the nonobese resistant (NOR) strain, which is a diabetes-resistant congeneric derivative of NOD/LtJ, having ~13% of its genome derived from C57BLKS/J (11). The NOR strain contains C57BLKS/J-derived DNA on distal chromosome 18 (12), which is consistent with our evidence supporting a role for this region in diabetes susceptibility. Interestingly, NOR also contains C57BLKS/J-derived proximal chromosome 18 DNA, to where Idd21.3 maps. Neither of these regions, however, segregate with diabetes in (NOD × NOR)F$_2$ (12).

Although a large number of loci have been implicated in NOD diabetogenesis, the number of Idd alleles is relatively limited in individual inbred strains (for example, an estimate of six non-MHC susceptibility genotypes is required for diabetes in NON, $F^{207} \times$ NOD)$F_2 \times$ NOD [13]), implying a relatively limited number of genetic checkpoints in the diabetogenesis of individual NOD mice. The actual genes determining diabetes in particular NOD crosses is determined by Idd allelic contributions from the inbred partner strain. Known Idd loci have been detected and confirmed largely as a result of genome-wide scan and congeneric analysis, with a limited number of comparison inbred strains (ABH, ALR, C57BL/6, C57L, C57BL/10, NOR, NON, PWK, and SJL), implying that the use of additional comparison strains in further genome-wide linkage scans would likely detect novel Idd loci. The current challenge is to identify the genes underlying known Idd loci and to further characterize their role in NOD diabetes etiology. Ultimately, a complete knowledge of genes able to regulate NOD diabetogenesis would aid interpretation of imminent genome-wide scans for genetic association to type 1 diabetes in humans. In the case of Idd21, we focused on the chromosome 18 (ABH × NOD)$F_2 \times$ NOD linkage (6) with the aim of using comparative mapping to enhance characterization of IDDM6.

The only autoimmune diabetes loci previously demonstrated to map to an orthologous region in human, mouse, and rat are within the MHC (IDDM1, Idd11/16, Iddm1, respectively). Here, we have demonstrated the presence of a second autoimmune diabetes locus colocalizing in all three species: IDDM6, Iddm3, and now Idd21.1–21.2. Further genetic localization of Idd21.1 and Idd21.2 could expedite positional cloning efforts within the orthologous IDDM6 region on human chromosome 18q12-q21.

**RESEARCH DESIGN AND METHODS**

NOD/MrcTak mice are derived from founders purchased from Taconic (Germantown, NY) in 1998 and NOD/LtSz-scid mice from founder mice purchased from The Jackson Laboratory (Bar Harbor, ME) in 2003. To create the NOD.ABH-(Chr18) subcongenic strains, male consomic NOD-Chr18$^{mmt}$ (NE6) mice (6) were backcrossed to NOD, male progeny backcrossed to NOD, and progeny screened with eight framework microsatellite markers. The eight congeneric lines (Table 1) were derived from four independent pairs of (NOD-Chr18$^{mmt}$ × NOD) × NOD backcross parents: the heterozygous recombinant founder NOD.ABH-(D18Mit8-D18Mit4) and NOD.ABH-(D18Mit207-D18Mit44) and NOD.ABH-(D18Mit124-D18Mit44) and NOD.ABH-(D18Mit235-D18Mit44) males (NE6) from a second pair; the heterozygous recombinant founder NOD.ABH-(D18Mit111-D18Mit44) and heterozygous founder NOD-Chr18$^{mmt}$ males (NE6) from a third pair; and the heterozygous founder NOD.ABH-(D18Mit64-D18Mit239) male (NE6) from a fourth pair. The recombinant mice were backcrossed to NOD and heterozy-
gous progeny intercrossed to produce the homozygous founder congenic lines (Fig. 1). A control consomic NOD-Chr18<sup>ABH</sup> (NES) strain was also bred. The integrity of the congenic strains was checked with an additional 21 markers (Fig. 1). To establish NOD.ABH-(D18Mit8-D18Mit4) (NE11), a homozygous NOD.ABH-(D18Mit8-D18Mit4) (NES) male was backcrossed to NOD, progeny were backcrossed two further times, and heterozygous males and females intercrossed to generate the homozygous line. Mice were housed in a specific-pathogen–free facility and fed a soy/wheat-based diet modeled on the U.S. Teklad diet RMH3500 but using New Zealand ingredients. The colony is specific-pathogen–free facility and fed a soy/wheat-based diet modeled on the

**Candidate gene position.** No single nucleotide polymorphism markers able to discriminate between NOD and ABH at Smad4 were identified (mCV23955461, mCV23955452, mCV23954417, and mCV23955841 [available at www.celera.com were tested]; thus, the exact position of Smad4 relative to the recombination event distinguishing NOD.ABH-(D18Mit207-D18Mit4) and NOD.ABH-(D18Mit8-D18Mit4) could not be determined. mCV23447010 and mCV23445638 were used to map Dcc in the Hdi21.2 interval and mCV2392724 to position Smad3 (Fig. 1).

**Assessment of diabetes.** Animals were classified as having diabetes when urinary glucose was at least 500 mg/dl (28 mmol/l). Cohorts of female mice were followed for diabetes development until 30 weeks of age. Incidence of diabetes in the University of Otago NOD colony has remained constant during the time period over which this work was done: incidence at 30 weeks in female NOD animals born in 2001 was 82.5% (n = 104), 86.2% in 2002 (n = 32), 87.1% in 2003 (n = 104), and 82.4% (n = 69) in 2004. There were no significant differences in any of the six pairwise comparisons between diabetes incidence curves (P = 0.24–0.88) (curves not shown).

**Histological analysis.** Female NOD and NOD.ABH-(D18Mit8-D18Mit4) mice were taken at 8–10 weeks of age and analyzed for pancreatic mononuclear cell infiltration (insulitis). Two 5-μm sections at least 200 μm apart were taken from each formalin-fixed and paraffin-embedded pancreas and stained with hematoxylin and eosin. All islets (average 15 per mouse) were scored as follows: islets completely free of infiltrate = 0, those with perivascular/periductal and peri-islet infiltration = 1 (peri-insulitis), those with <25% of the islet infiltrated = 2 (mild insulitis), and islets with >25% infiltration = 3 (severe insulitis). The total score for each mouse was calculated and mean scores for each strain compared using a one-tailed t test assuming equal variance.

**Adoptive transfer.** Splenocytes were isolated from 8- to 10-week-old female NOD and NOD.ABH-(D18Mit8-D18Mit4) mice, and 5 × 10<sup>6</sup> splenocytes were injected intravenously into age-matched NOD/LtSz-scid female mice.

**ACKNOWLEDGMENTS**

J.E.H.-M. was supported by a New Zealand Lottery Health Postgraduate Scholarship. The work was supported by the New Zealand Lottery Health and the New Zealand Health Research Council.

We would like to acknowledge the expert staff at the University of Otago Department of Laboratory and Animal Sciences. We thank the anonymous reviewers for their input into this manuscript.

**REFERENCES**


