Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 5898–5902, June 1994
Immunology

Genetic and physiological association of diabetes susceptibility with raised Na^+/H^+ exchange activity

(autoimmunity/type I diabetes/nonobese diabetic mice/antiporter/gene mapping)

GRANT MORAHAN*†, PETER MCCLIVE*, DEXING HUANG*, PETER LITTLE‡, AND ALAN BAXTER*§

*The Walter and Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital, Victoria 3050, Australia; and ‡The Baker Medical Research Institute, Prahran, Victoria 3181, Australia

Communicated by J. F. A. P. Miller, February 9, 1994 (received for review November 25, 1993)

ABSTRACT **Insulin-dependent diabetes mellitus is a multigenic autoimmune disease, for which one of the best animal models is the nonobese diabetic (NOD) mouse strain. In both humans and NOD mice, major histocompatibility complex genes are implicated as risk factors in the disease process. Other susceptibility genes are also involved, and a number have been mapped in the mouse to specific chromosomal locations. To identify further susceptibility genes, diabetic backcross mice, produced after crossing NOD/Lt to the nondiabetic strains SJL and C57BL/6 (B6), were examined for markers not previously associated with disease susceptibility. Linkage was found to loci on chromosomes 4 and 14. Of the candidate loci on chromosome 4, the gene encoding the Na^+/H^+ exchanger-1, *Nhe-1*, was the most likely, since the NOD allele was different from that of both nondiabetic strains. NOD lymphocytes were found to have a higher level of Na^+/H^+ exchange activity than lymphocytes from either B6 or SJL mice. Since the chromosome 4 susceptibility gene is recessive, the B6 allele should prevent diabetes. This prediction was tested in fourth-generation backcross mice, selected for retention of the B6 allele at *Nhe-1*. Mice homozygous for *Nhe-1* developed diabetes after cyclophosphamide treatment, but heterozygotes were largely protected from disease. These results implicate the Na^+/H^+ exchanger (antiporter) in the development of type 1 diabetes and may provide a screening test for at-risk individuals as well as offering prospects for disease prevention.**

Insulin-dependent diabetes mellitus is characterized by lymphocytic infiltration of the pancreatic islets, in which T lymphocytes mediate the destruction of insulin-producing β cells. Perhaps the best animal model of insulin-dependent (or type 1) diabetes is the nonobese diabetic (NOD) mouse strain (1), which has been the subject of many immunological and genetic studies aimed at defining the properties that contribute to disease susceptibility. In both humans and NOD mice, genes mapping to the major histocompatibility complex are important in the disease process (2). In NOD mice, this gene conferring insulin-dependent diabetes susceptibility is termed *Idd-1*.

Despite the importance of the major histocompatibility complex to the disease process, other genes also contribute. Their nature is not well defined in the human disease, but genetic analyses of NOD mice have revealed that several genes are involved. Some of these have been mapped to specific chromosomal locations, although their products have not been identified (3–6). These diabetes susceptibility genes (DSG) are numbered from *Idd-2*, which is linked to the *Thy-1* gene on chromosome 9 (7). Todd *et al.* (3) defined the *Idd-3* and *Idd-4* loci on chromosomes 3 and 11, which influence occurrence of diabetes and time of onset of disease. A further gene on chromosome 1 was implicated in periinsulitis and the lymphocytic infiltration observed in various other organs of NOD mice, such as the salivary glands (4, 8). Another gene was mapped to chromosome 15 by using an interspecies backcross (6). In a report to appear elsewhere, we show that diabetes susceptibility alleles are shared by strains of mice that do not develop diabetes (A.B., unpublished data). This study also suggested that there were additional DSG that had not been defined previously. We now report data that map previously undisclosed DSG to mouse chromosomes 4 and 14.

MATERIALS AND METHODS

Mice. NOD/Lt, C57BL/6 (B6), and SJL mice were obtained from the Walter and Eliza Hall Institute specific pathogen-free facilities and maintained in a conventional mouse colony. Diabetic *H-2*^{g7}-homozygous female mice from the NOD/Lt × (B6 × NOD/Lt) and NOD/Lt × (SJL × NOD/Lt) backcrosses were obtained as will be described elsewhere (A.B., unpublished data). Briefly, first generation backcross females, selected for the absence of H-2 antigens of the nondiabetic strain, were monitored for the development of diabetes by testing blood glucose levels at 2-week intervals. BC1 males were crossed to NOD/Lt and progeny were screened for heterozygosity at *Nhe-1*, the gene encoding the Na^+/H^+ exchanger 1 (NHE-1). Further backcrossing and selection produced an incipient NOD/*Nhe-1* congenic line.

Genetic Analyses. Diabetic mice from each backcross were typed as described at loci in which NOD alleles were shown to differ from both B6 and SJL alleles (9). Restriction fragment length polymorphisms were typed by hybridization to nylon membranes (Amersham) according to the manufacturer's recommendations. Microsatellite markers were analyzed (10) by using primers obtained from Research Genetics (Huntsville, AL).

To define the chromosomal localization of putative DSG, the backcross panels were typed for inheritance of alleles from the nondiabetic strain at further loci on chromosomes 4 and 14. Markers were chosen according to their position on distal chromosome 4 (11) or on the corresponding region of the human genome—i.e., chromosome 1p; these loci have since been placed on the mouse map by typing recombinant inbred strains (P.M., unpublished data). The order of microsatellite markers and their position relative to other chromosome 4 loci were taken from the data of Dietrich *et al.* (10) and of P.M. (unpublished data). Chromosome 14 marker positions were from refs. 10 and 11 and from the Whitehead/MIT Center for Genome Research "Genetic Map of the Mouse" Database Release 3, 1993. Probes detecting previously un-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: DSG, diabetes susceptibility gene(s); NOD, nonobese diabetic; B6, C57BL/6; pH_i, intracellular pH; NHE-1, Na^+/H^+ exchanger 1.

†To whom reprint requests should be addressed.
§Present address: Department of Pathology and Immunology, Cambridge University, Cambridge, United Kingdom.

described polymorphisms between NOD and the nondiabetic strains were *Gcsfr* and *Gcsfr-rs* (N. Gough, personal communication), *Lck* (12), *Lap-18* (13), *ang* (14), *Tcra-Vα8* (15), and *Rb-1* (16). *Nhe-1* alleles were typed by using a human cDNA probe (17) as described (18).

Measurement of Na^+/H^+ Exchange Activity. Pooled spleen and lymph node cell suspensions from B6, SJL, and nondiabetic NOD mice were erythrocyte-depleted and loaded with the pH-sensitive dye BCECF [2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein] as described (19). Fluorescence measurements were made with a dual wavelength fluorimeter CM-2 (Spex Industries, Metuchen, NJ). Cells were acidified for experiments and for construction of the calibration curve by the "nigericin-clamp" technique; all measurements were made in cells from which nigericin was removed by washing with solutions containing bovine serum albumin. A 100-μl aliquot of each cell suspension was added rapidly to 2.9 ml of stirred physiological saline solution (135 mM NaCl/5 mM KCl/1.8 mM $CaCl_2$/0.8 mM $MgSO_4$/5.5 mM glucose/10 mM Hepes, pH 7.35 at 37°C) in a thermostated cuvette at 37°C. Kinetic rates were determined from the initial phase (15–20 s) of alkalinization. All data were derived from at least two different lymphocyte preparations and were analyzed by Student's *t* test and ANOVA.

As antiporter measurements can be complicated by differences in buffer capacity, this parameter was determined (20) at a nominal pH value of 6.49 (the midpoint of the experimental range). NOD lymphocytes had a buffer capacity of 64.2 ± 9.3 mmol per liter per pH unit (n = 4) at an experimentally determined intracellular pH (pH_i) of 6.44 ± 0.03; B6 lymphocyte buffer capacity was 44.2 ± 9.2 mmol per liter per pH unit (n = 4; pH_i of 6.52 ± 0.03). These values were not significantly different ($P > 0.1$; unpaired Student's *t* test) and were similar to others in the literature.

Induction of Diabetes. Cyclophosphamide was used to induce diabetes (21, 22) at a dose of 350 mg/kg of body weight in mice of the fourth or sixth backcross generations of the incipient NOD/*Nhe-1*^{b} congenic strain. Diabetes status was monitored over 2–4 weeks by testing either random blood glucose or urinary glucose levels.

RESULTS

Identification of Previously Unreported DSG. To detect genes that could confer disease susceptibility, backcross mice (produced after crossing NOD/Lt to the nondiabetic strains SJL and B6) were examined for inheritance of markers on chromosomes not associated with susceptibility. Female mice were selected for homozygosity of NOD *H-2*, which is necessary for development of diabetes (2). Over 200 of these *H-2*^{g7}-homozygous backcross mice were monitored for diabetes. Ten diabetic mice from each panel were assessed for inheritance of markers linked to *Idd-2–Idd-7* (A.B., unpublished data) and were typed at loci on other chromosomes (Table 1).

As expected, most of the loci tested showed no evidence of linkage to disease. However, significant homozygosity of NOD alleles was found at loci on chromosomes 4 and 14. To evaluate this observation, 30 nondiabetic mice from the B6 backcross were chosen at random and typed at these loci. The results did not deviate from that expected from Mendelian inheritance: 14 of 30 were heterozygous at *D4Mit71*, while 17 of 30 were heterozygous at *D14Mit11*. Therefore, there was no selective disadvantage of heterozygosity of NOD alleles at these loci. These results suggest linkage of potential DSG to markers on chromosomes 4 and 14.

Mapping of the DSG on Chromosome 4. A number of genes on distal chromosome 4 could potentially be involved in aberrant immune responses. To exclude some of these, the backcross panels were typed at flanking markers on this chromosome (Table 2). All but one of the B6 backcross diabetic mice were homozygous at the chromosomal region from *Lck* to *Lap-18*. Crossovers occurred in this panel between *Lck* (12) and *D4Mit72* and between *Lap-18* and *D4Mit13* (10). These results exclude as candidate susceptibility genes the *Gcsfr*, c-*jun*, and *TNFR2* loci as well as *Glut-1* and *Mls-2*, both of which are proximal to *Gcsfr*. Instead, the most likely location of a chromosome 4 DSG is between *D4Mit72* and *D4Mit13*. This distance is less than 10 centimorgans (cM) estimated from typing recombinant inbred strains (not shown). Similar results were obtained in the SJL panel: all but one of the mice were homozygous at *Nhe-1* on distal chromosome 4, while another two were heterozygous at the most proximal marker typed, c-*jun*. Similar analyses suggested that the most likely location for the DSG on chromosome 14 is proximal to *D14Mit45* (Table 2).

Four genes mapping to the *D4Mit72–D4Mit13* interval were considered as candidates for the chromosome 4 DSG: *Lck* (12), involved in T-cell activation; *Nhe-1* (18), which

Table 1. Genotype of diabetic backcross mice at polymorphic markers on chromosomes not previously known to contain diabetes susceptibility genes

		B6 allele											SJL allele											
		Presence in backcross mouse number										No.	Presence in backcross mouse number										No.	
Locus	Chr.	7	19	24	36	48	68	72	94	98	138	het.	16	23	29	32	47	50	65	84	86	92	het.	χ^2
D4Mit11	4					*		*				2								*			1	8.46
D5Mit16	5		*		*	*	*				*	5			*			*	*	*	*		5	0.05
D8Mit15	8	*		*			*		*			4	*	*	*	*				*			5	0.05
D10Mit20	10	*	*			*		*		*		5	*			*							2	1.25
D12Mit4	12	*	*			*	*	*		*		6	*		*			*	*	*	*	*	7	1.25
D13Mit9	13	*	*	*		*	*	*	*	*		8	*		*	*			*	*			5	1.25
D14Mit11	14		*				*					2							*				1	8.46
D16Mit11	16			*		*				*	*	4	*			*	*		*			*	5	0.05
D18Mit12	18	*	*	*						*		4		*		*		*		*	*		5	0.05
GCSFR-rs	19			*	*	*	*	*			*	6		*	*				*			*	4	0.05

Ten diabetic mice (each numbered) from each backcross panel were typed for inheritance of B6 or SJL alleles of loci at which NOD mice had been shown (9) to differ from both of these strains. Presence of nondiabetic strain alleles is indicated by an asterisk. Linkage to previously described DSG on chromosomes 1, 3, 6, 7, 9,11, 15, and 17 and to the *GAD* locus on chromosome 2 will be analyzed elsewhere (A.B., unpublished data). There was no significant difference between the backcross panels for any of these markers by Fischer's exact test, so these results were pooled. Results of Yates' corrected χ^2 test are shown. χ^2 values > 3.84 indicate significance at the $P = 0.05$ level; and values > 10.83, at the 0.001 level. Although usually corrections are not made for multiple tests (e.g., ref. 6), such an allowance may be made by dividing α by the number of tests; in this case, for testing linkage to 10 markers, a χ^2 value > 7.88 indicates significant linkage ($P < 0.05$). Chr., chromosome; No. het., number of heterozygotes.

Table 2. Mapping of diabetes susceptibility genes on mouse chromosomes 4 and 14

		B6 allele											SJL allele										
		Presence in backcross mouse number										No.	Presence in backcross mouse number									No.	
Locus	Chr.	7	19	24	36	48	68	72	94	98	138	het.	16	23	29	32	47	50	65	84	86	92	het.	χ^2
c-jun	4	=	=	=	=	=	=	=	=	=	=						*	*		*			3	
D4Mit11	4					*		*				2								*			1	8.46
Gcsfr	4					*		*				2	=	=	=	=	=	=	=	=	=	=		
D4Mit72	4					*		*				2								*			1	8.46
Lck	4					*						1	=	=	=	=	=	=	=	=	=	=		
D4Mit71	4					*						1	=	=	=	=	=	=	=	=	=	=		
Nhe-1	4					*						1								*			1	11.3
Lap-18	4					*						1	=	=	=	=	=	=	=	=	=	=		
D4Mit13	4				*	*						2	=	=	=	=	=	=	=	=	=	=		
TNFR2	4				*	*						2	=	=	=	=	=	=	=	=	=	=		
D14Mit1	14		*				*					2	=	=	=	=	=	=	=	=	=	=		
D14Mit40	14		*				*					2							*				1	8.46
D14Mit11	14		*				*					2	=	=	=	=	=	=	=	=	=	=		
D14Mit44	14		*				*					2							*				1	8.46
D14Mit45	14		*				*				*	3			*				*				2	4.05
D14Mit18	14						*				*	2	=	=	=	=	=	=	=	=	=	=		
D14Mit4	14						*				*	2	=	=	=	=	=	=	=	=	=	=		
Ang	14						*				*	2	=	=	=	=	=	=	=	=	=	=		
Tcra	14						*				*	2	=	=	=	=	=	=	=	=	=	=		
D14Mit30	14						*				*	2	=	=	=	=	=	=	=	=	=	=		
Rb-1	14						*				*	2	=	=	=	=	=	=	=	=	=	=		

Diabetic backcross mice shown in Table 1 were analyzed for inheritance of markers on chromosomes 4 and 14. The presence of nondiabetic strain alleles is indicated by an asterisk; an equal sign (=) indicates no polymorphism found between the nondiabetic strain and NOD. Results of Yates' corrected χ^2 test are shown where both B6 and SJL differ from NOD. Chr., chromosome; No. het., number heterozygous alleles.

regulates intracellular pH, cell volume, and other aspects of maintenance of cellular viability, particularly after activation (17, 23); *Lap-18*, the mouse homologue of a human gene encoding a phosphoprotein in leukemia cells (13); and *ClqC* (ref. 24; P.M., unpublished data), which directs the synthesis of the B chain of the major complement component. A candidate gene should vary between NOD and both of the nondiabetic strains, since linkage to chromosome 4 was observed in both backcross panels. Polymorphisms were indeed found (Fig. 1), but NOD and SJL strains shared alleles at three of these loci, consistent with other observations of these surprisingly closely related strains (9). Only the *Nhe-1* locus was variable between NOD and the other strains; indeed, this locus has at least three alleles. Naturally, the inability to find polymorphisms between NOD and SJL at the other loci does not prove that they are invariant, but even sequence differences, were they to be found, may not be biologically relevant. Therefore, demonstration of functional differences between alleles of these strains is crucial.

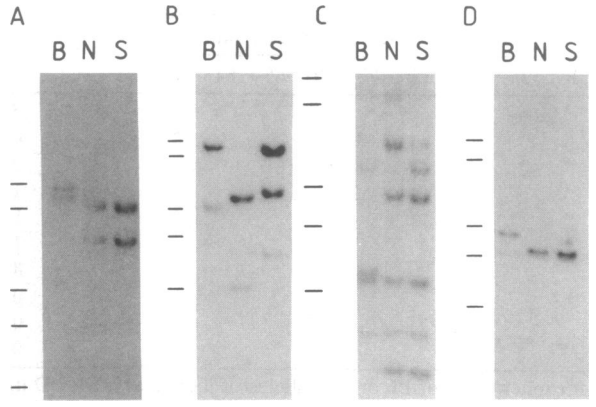

FIG. 1. Analysis of candidate genes. Polymorphisms were detected in DNA isolated from B6 (lanes B), NOD/Lt (lanes N), and SJL (lanes S) mice by using probes for the following genes on distal mouse chromosome 4: *Lck* (12) (*A*); *Nhe-1* (18) (*B*); *Lap-18* (13) (*C*); and *ClqC* (24) (*D*). The map positions of *Lap-18* and *Clq* are to be described elsewhere (P.M., unpublished data).

Na^+/H^+ Exchange Activity Varies Between NOD and the Nondiabetic Strains. The product of the *Nhe-1* gene is responsible for the maintenance of pH_i by transferring Na^+ and H^+ ions across the cell membrane (23). To test whether there was a physiological difference in the activity of the NHE-1 molecule, NOD and B6 lymphocytes were examined for their ability to regulate pH_i (Fig. 2). NOD lymphocytes had a higher resting intracellular pH than did B6 cells (Fig. 2*A*). Furthermore, antiporter activity (indicated by recovery from induced intracellular acidosis) was higher in cells from NOD mice than in those from either B6 or SJL mice (Fig. 2 *B* and *C*). Therefore, *Nhe-1*, differing in both genetics and function between NOD and the nondiabetic strains, is the best candidate for a DSG on mouse chromosome 4.

Role of the *Nhe-1*-Linked DSG in Cyclophosphamide-Induced Diabetes. To confirm that the DSG on chromosome 4 was involved in diabetes susceptibility, we started production of *Nhe-1* congenic strains by repeated backcrossing to the NOD/Lt strain, selecting at each generation for the presence of the B6 allele (i.e., $Nhe\text{-}1^b$; ref. 18) or the *D4Mit71* microsatellite, which is within 1 centimorgan (P.M., unpublished data). After four backcross generations, mice were homozygous for NOD alleles at *H-2*, at markers linked to *Idd-2–Idd-5*, and at the chromosome 14 DSG mapped in Table 2. The ability of the B6 allele of *Nhe-1* to offer protection from diabetes was tested by cyclophosphamide treatment, which accelerates the onset of diabetes in NOD mice (21, 22). Mice were monitored up to 4 weeks after treatment. Table 3 compares the diabetes incidence in NOD/$Nhe\text{-}1^a$ homozygous and NOD/$Nhe\text{-}1^{a/b}$ mice of the fourth backcross generation. Most of the *Nhe-1* homozygous mice developed diabetes (Table 3) and did so with a frequency similar to that observed in cyclophosphamide-treated NOD/Lt mice in our hands (not shown). In contrast, their heterozygous littermate mice were largely protected from cyclophosphamide-induced disease (Table 3). Therefore, homozygosity of NOD alleles at *Nhe-1* or a very closely linked

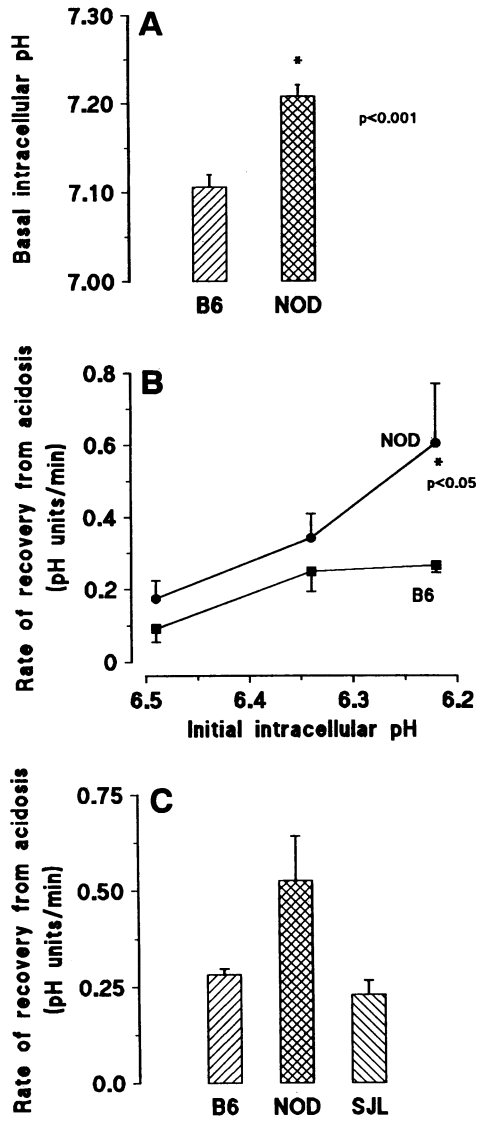

FIG. 2. Comparison of Na^+/H^+ exchange activity. (*A*) Measurement of resting pH_i in B6 [*n* (number of measurements) = 5] and NOD (*n* = 6) lymphocytes. (*B*) Rates of pH_i recovery from induced acidosis at differing pH. Four determinations were made over the initial 20 s after acidification for each strain at each point (i.e., pH 6.49, 6.34, and 6.22). (*C*) Maximal rate of pH_i recovery in B6 (*n* = 8), NOD (*n* = 6), and SJL (*n* = 5) lymphocytes. Asterisks in *A* and *B* indicate significant values ($P < 0.001$ in *A*; $P < 0.05$ in *B*).

locus are required for diabetes development (in this case, after cyclophosphamide treatment), consistent with a recessive DSG. Similar results were obtained with heterozygous mice from the sixth backcross generation, at which stage the mice were homozygous for NOD alleles at loci distal to *D4Mit69* (not shown) which is 1 centimorgan distal to *Nhe-1* (P.M., unpublished data).

Table 3. Diabetes resistance in NOD/*Nhe-1*^{b} congenic mice

Presence of *Nhe-1*^{b} allele	Diabetic mice		
	No. diabetic/total	%	*P*
−	9/13	69	
+	3/12	25	<0.05

Fourth generation backcross mice were typed for inheritance of the B6 allele of *Nhe-1* or the closely linked microsatellite marker *D4Mit71*. Cyclophosphamide was used to induce diabetes (21, 22). Diabetes status was monitored over 2–4 weeks by testing both random blood glucose and urinary glucose levels. Results of two separate experiments were combined and analyzed by Fischer's exact test.

DISCUSSION

Evidence is presented here of previously unreported diabetes susceptibility genes mapping to distal mouse chromosome 4 and to proximal chromosome 14. It was possible to exclude a number of loci on chromosome 4 that otherwise may have been considered for playing a role in autoimmunity—e.g., genes encoding glucose transporter 1 and receptors for granulocyte colony-stimulating factor (G-CSF) and tumor necrosis factor (TNF); oncogene c-*jun*; and the *Mls-2* locus. The best candidate for the DSG on distal chromosome 4 was *Nhe-1*. Of the known genes mapping to the relevant region, it alone displays molecular variation between NOD mice and each of the other strains used in this study (Fig. 1). Furthermore, this genetic variation has biological relevance: both basal pH_i and Na^+/H^+ exchange activity, mediated by the product of this locus, are higher in NOD mice than in either B6 or SJL mice (Fig. 2).

Although the mapping panel we used was relatively small, the results nevertheless implicated unreported disease genes; we welcome investigations of these genes in larger studies. Recently, other DSG (*Idd-8* and *-9*) were mapped to chromosomes 4 and 14 (25). However, the chromosome 4 DSG (*Idd-9*) appears more distal than *D4Mit13* and consequently cannot be *Nhe-1*. Furthermore, the incipient congenic strain does not retain B6 alleles distal to *D4Mit13* (not shown). Therefore, we provisionally designate the DSG linked to *Nhe-1* as *Idd-11*. Although the chromosome 14 locus mapped here is near *Idd-8* (25), it confers susceptibility, whereas the NOD allele of *Idd-8* was described as having a protective effect (3, 25). Further characterization of this gene, provisionally designated *Idd-12*, is needed.

The question may be asked as to why *Nhe-1* was not identified previously as a DSG. Susceptibility was not found to be linked to chromosome 4 in a larger study (3). However, it is significant that this investigation used B10-*H-2*^{g7} as a nondiabetic partner. Although the B6 and C57BL/10 (B10) strains are often considered virtually identical, we found they differed at a number of loci on chromosome 4 (26). Moreover, B10 strains had the same *Nhe-1* allele as in NOD mice (26). This observation both makes it unlikely that homozygosity of NOD alleles in this region of chromosome 4 would have been selected (3) and strengthens the case for the involvement of *Nhe-1* in diabetes susceptibility.

The protection from cyclophosphamide-induced diabetes offered by heterozygosity of *Nhe-1* may be contrasted with that of the only previously proven DSG, which encodes the unique NOD class II molecule (2). NOD mice transgenic for other class II alleles, such as *I-A*^{k}, normally do not develop spontaneous diabetes. However, NOD/*I-A*^{k} transgenic mice are not protected from developing cyclophosphamide-induced diabetes (27). The ability of the B6 ("diabetes resistance") allele of *Nhe-1*, present in heterozygous congenic mice, to prevent even the powerful diabetes-inducing activity of cyclophosphamide indicates that this gene is important in the diabetogenic process.

The role played by *Nhe-1* in the development of diabetes in NOD mice is unclear because of its ubiquitous cellular expression and involvement in many cellular processes (23). Given the importance of pH_i, it was surprising to find such a clear difference in basal pH between cells from NOD and other strains. This difference could be attributed to increased levels of Na^+/H^+ exchange activity, mediated by NHE-1. Recessive gene products usually are defective in some activity; the NOD allele of *Nhe-1* is unusual in that it confers a higher level of activity.

Significantly, high NHE-1 activity may also be involved in human diabetes. Antiporter levels are often elevated in cells obtained from diabetic patients (e.g., refs. 28–30). These studies may be complicated by the observation that diabetes *per se* may lead to raised antiporter levels—e.g., drug-induced diabetic rats have elevated antiporter activity (31). Such complications may be avoided by analysis of cultured cells. Fibroblasts from type I diabetes patients with nephropathy had high antiporter levels; this was taken as evidence that such patients were predisposed to hypertension and hence nephropathy (32). In contrast, the present results implicate high antiporter activity as playing a causal role in diabetes development, changing the focus of the involvement of *Nhe-1* to its predisposing first to diabetes and subsequently to nephropathy.

Earlier studies that showed elevated antiporter levels in diabetic patients without proteinuria (28, 29) should be confirmed by analyses of cultured cells. However, it is clear that the disease of NOD mice may share similar etiology with at least a subset of type I diabetes patients. Just as NOD mice and Caucasoid diabetic patients have similar unique MHC genes, high levels of antiporter activity may also be shared by certain susceptible individuals in both species. If so, this provides the basis for a genetic screening test to identify those who are at risk of developing this autoimmune disease and its most severe consequences.

We thank Cheryl Augustine for excellent technical assistance and Dr. Alex Bobik (Baker Medical Research Institute) for helpful discussions. We are also grateful to those investigators who generously provided probes. This work was supported by the National Health and Medical Research Council of Australia, the Victorian Health Promotion Foundation, the National Heart Foundation of Australia, and the Alfred Hospital Research Trust. A.B. is a recipient of a National Health and Medical Research Council C. J. Martin Fellowship.

1. Makino, S., Kunimoto, I. K., Muraoka, Y., Mizushima, Y., Katagiri, K. & Tochino, Y. (1980) *Exp. Anim.* **29**, 1–3.
2. Todd, J. (1990) *Immunol. Today* **11**, 122–129.
3. 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.
4. Cornall, R. J., Prinsi, J. B., Todd, J. A., Pressey, A., DeLarato, N. H., Wicker, L. S. & Peterson, L. B. (1991) *Nature (London)* **353**, 262–265.
5. Garchon, H.-J. (1992) *Curr. Opin. Immunol.* **4**, 716–722.
6. De Gouyon, B., Melanitou, E., Richard, M., Requarth, M., Hahn, I., Guenet, J., Demenais, F., Julier, C., Lathrop, G., Boitard, C. & Avner, P. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 1877–1881.
7. Prochazka, M., Leiter, E. H., Serreze, D. V. & Coleman, D. L. (1987) *Science* **237**, 286–289.
8. Garchon, H. J., Bedossa, P., Eloy, L. & Bach, J. F. (1991) *Nature (London)* **353**, 260–262.
9. McClive, P., Baxter, A. G. & Morahan, G. (1994) *Immunol. Cell Biol.*, **72**, 137–142.
10. Dietrich, W., Katz, H., Lincoln, S. E., Shin, H. S., Friedman, J., Dracopoli, N. & Lander, E. S. (1992) *Genetics* **131**, 396–423.
11. Elliott, R. (1992) *Mouse Genome* **90**, 599–669.
12. Huppi, K., Mock, B. A., Shricker, P., D'Hoostelaere, P. & Potter, M. (1988) *Curr. Top. Microbiol. Immunol.* **137**, 276–288.
13. Ferrari, A. C., Seuanez, H. N., Hanash, S. M. & Atweh, G. F. (1990) *Genes Chromosomes Cancer* **2**, 125–129.
14. Steinhelper, M. E. & Field, L. J. (1992) *Genomics* **12**, 177–179.
15. Morahan, G., Hoffman, M. & Miller, J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 11421–11425.
16. Stone, J., Crosby, J., Kozak, C., Schievella, A., Bernards, R. & Nadeau, J. (1989) *Genomics* **5**, 70–75.
17. Sardet, C., Franchi, A. & Pouyssegur, J. (1989) *Cell* **56**, 271–280.
18. Morahan, G. & Rakar, S. (1993) *Genomics* **15**, 231–232.
19. Carr, P., Taub, N., Watts, G. & Poston, L. (1993) *Hypertension* **21**, 344–352.
20. Roos, A. & Boron, W. (1981) *Physiol. Rev.* **61**, 289–434.
21. Harada, M. & Makino, S. (1984) *Diabetologia* **27**, 604–606.
22. Charlton, B., Bacelj, A., Slattery, R. & Mandel, T. (1989) *Diabetes* **38**, 441–447.
23. Wakabayashi, S., Sardet, C., Fafournoux, P., Counillon, L., Meloche, S., Pages, G. & Pouyssegur, J. (1992) *Rev. Physiol. Biochem. Pharmacol.* **119**, 157–185.
24. Petry, F., Reid, K. B. & Loos, M. (1991) *J. Immunol.* **147**, 3988–3993.
25. Ghosh, S., Palmer, S., Rodrigues, N., Cordell, H., Hearne, C., Cornall, R., Prins, J.-B., McShane, P., Lathrop, G., Peterson, L., Wicker, L. & Todd, J. (1993) *Nature Genet.* **4**, 404–409.
26. McClive, P., Huang, D. & Morahan, G. (1994) *Immunogenetics*, **39**, 286–288.
27. Slattery, R., Miller, J., Heath, W. & Charlton, B. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 10808–10810.
28. Salles, J., Ser, N., Fauvel, J., Couvaras, O., Bouissou, F., Ghisolfi, J., Barthe, P. & Chap, H. (1991) *J. Hypertens. Suppl.* **9**, S222–S223.
29. Halkin, A., Benjamin, N., Doktor, H., Todd, S., Viberti, G. & Ritter, J. (1991) *Clin. Sci.* **81**, 223–232.
30. Ng, L. & Davies, J. (1991) *Kidney Int.* **41**, 872–876.
31. Boyarsky, G., Rosenthal, N., Barrett, E. & Boron, W. (1991) *Am. J. Physiol.* **363**, C167–C175.
32. Davies, J., Ng, L., Kofoed-Enevoldsen, A., Li, L., Earle, K., Trevisan, R. & Viberti, G. (1992) *Kidney Int.* **42**, 1184–1190.