The Influence of Genetic Background on the Susceptibility of Inbred Mice to Streptozotocin-induced Diabetes

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SUMMARY

Multiple low-dose injections of streptozotocin (STZ) induce a delayed but progressively increasing state of hyperglycemia in mice. Different inbred strains of mice show different susceptibility to this treatment. We examined whether genetic factors associated with the *H-2* complex influence the susceptibility or resistance, using a selected group of 12 inbred and 5 congenic resistant strains of mice. We found that different congenic strains differed significantly in their susceptibility to STZ-induced diabetes, suggesting that *H-2*-associated genes do influence the susceptibility. However, at least some inbred strains sharing the same *H-2* haplotype also differed in their susceptibility, indicating that genes outside the *H-2* complex may also affect the susceptibility. Therefore, there appear to be at least two genes, one within and one or more outside the *H-2* complex, that determine the susceptibility to multiple low doses of STZ. DIABETES *33*:567–571, June 1984.

Evidence has accumulated that type I (insulin-dependent) diabetes mellitus in humans may be an autoimmune disease (reviewed in ref. 1). Particularly significant in this regard is the finding that specific alleles from the *HLA* complex, especially the *HLA-D* locus, are associated with an increased risk to type I diabetes, while other alleles are associated with a reduced risk.[2]

An experimental model system of type I diabetes was developed by Like and Rossini.[3–5] Using mice, they showed that a single high dose of streptozotocin (STZ, 200 mg/kg body wt) caused severe beta cell destruction and permanent hyperglycemia. In contrast, injection of the same total dose divided into five equal low doses (40 mg/kg/day) induced a slow, progressive hyperglycemia accompanied by lymphocytic infiltration of the pancreatic islets. Both the hyperglycemia and lymphocytic infiltration could be partly prevented by rabbit antisera against mouse lymphocytes.[5] Our laboratory has shown subsequently that the induction of hyperglycemia by the multiple low-dose procedure, but not the single high-dose procedure, was etiologically mediated by host T-cell-dependent functions.[6,7] These observations strongly suggested that autoimmune processes may be involved in the induction of progressive hyperglycemia by STZ.

If host autoimmunity indeed plays a role in this experimental disease, then host genes associated with the major histocompatibility complex may be expected to have a strong influence on susceptibility or resistance to it. Several previous investigators examined this question. Based on a study of 8 inbred strains of mice, Rossini and co-workers reported that susceptibility to diabetes as induced by multiple subdiabetogenic doses of STZ differed among strains with identical *H-2* haplotypes.[8] It was concluded, therefore, that *H-2*-associated genes were not the major determinants of susceptibility. Subsequently Kiesel et al. reported that the *H-2* gene complex did have a modulating effect.[9,10] Their study of five congenic resistant strains of mice appeared to suggest to the authors that the $H\text{-}2^s$ allele conferred relative resistance to STZ-induced hyperglycemia while the $H\text{-}2^k$ allele conferred sensitivity. Mice with the *H-2* haplotypes *d, b,* and *a* were moderately susceptible. In contrast, Kromann et al.[11] examined four congenic resistant strains and found no *H-2* influence. In an unrelated study of 19 strains of mice for susceptibility to diabetes induced by the EMC virus, Boucher and colleagues reported that five strains representing the haplotypes *d, s, a,* and *b* were relatively more susceptible, but three other strains representing *b, d,* and *k* were resistant,[12] indicating that susceptibility to the virally induced diabetes was not primarily determined by the *H-2* complex.

To resolve the discrepancies in previous studies and to examine further the possible role of *H-2*-associated host genetic factors in STZ-induced diabetogenesis, we tested a

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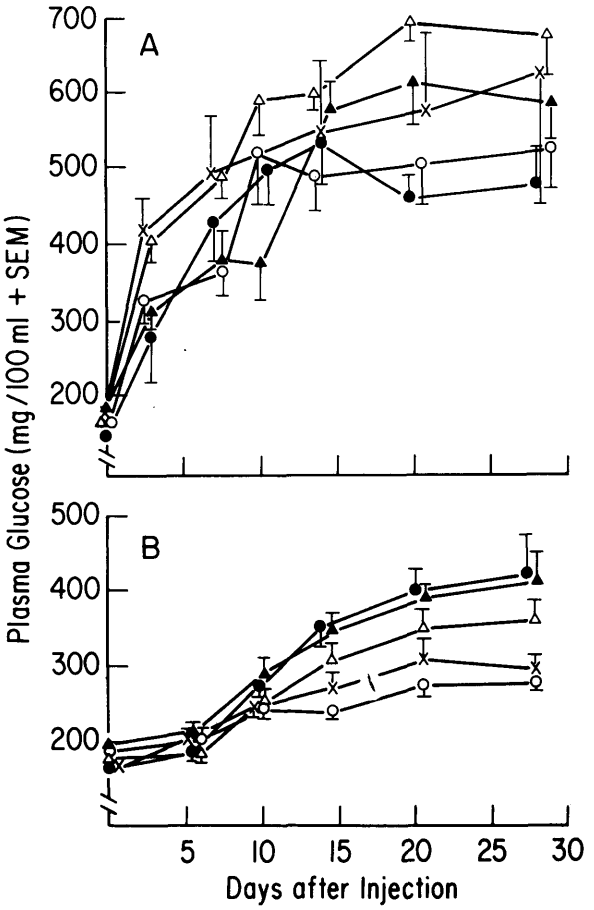

FIGURE 1. Influence of *H-2* haplotypes on induction of hyperglycemia by STZ. Mice of 5 congenic resistant strains in the BALB background were injected with (A) a single "high dose" of STZ (200 mg/kg body wt) on day 0, or (B) multiple "low dose" (40 mg/kg body wt/day) for 5 consecutive days on days 0–4, and changes in nonfasting glucose levels were measured for the following 28 days. Vertical bars represent standard error of the mean (SEM). —△—△— BALB.K (*H-2*^{k}); —X—X— BALB.5R (*H-2*^{i}); —▲—▲— BALB/cAn (*H-2*^{d}); —○—○— BALB.B (*H-2*^{b}); and —●—●— BALB.G (*H-2*^{g}).

selected group of inbred strains and congenic resistant strains of mice under experimental conditions established earlier. Since environmental as well as genetic factors have been shown to influence susceptibility to STZ,[7,13] we attempted to minimize the environmental variables in our study by using congenic strains that were bred and maintained in the laboratory of one of us (F.L.), and carried out all tests under uniform conditions previously used in our experimental series.[6,7,14]

MATERIALS AND METHODS

Mice. Male congenic resistant mice (BALB/cAn, BALB.B, BALB.G, BALB.K, and BALB.5R) as well as the inbred strain C3H/An were produced and maintained in a breeding colony in the laboratory of one of us (F.L.). The animals were 6–12 wk old at the start of each experiment. Male mice of the other inbred strains were obtained from Jackson Laboratory, Bar Harbor, Maine. All mice were housed for at least 1 wk in our mouse room (S.S.) before they were injected with STZ, so that any differences in glucose metabolism due to dietary variables would be minimized or eliminated. Where appropriate, procedures that we have used in our previous experiments with BALB/cBOM nude mice were employed throughout.[6,7]

Streptozotocin. Streptozotocin (STZ, mixed anomer, Sigma) was dissolved in sterile 0.1 M sodium citrate buffer, pH 4.5, and injected intraperitoneally into mice within 5 min after preparation.

Determination of plasma glucose levels. To minimize metabolic variability, all blood samples (80 μl) were collected between 9 a.m. and 11 a.m. from the paraorbital venous plexus of nonfasting animals using heparinized capillary tubes, and plasma glucose levels were measured with the Beckman Glucose Analyzer 2 by the glucose-oxidase method as previously described.[6]

RESULTS

Susceptibility to STZ-induced diabetes was first evaluated by injecting mice with either the "standard single high dose" (200 mg/kg body wt, once) or "standard multiple low doses" (40 mg/kg/day for 5 consecutive days). Control mice received only the solvent (citrate buffer). The development of hyperglycemia was monitored for 28 days after the injections by measuring the change in blood glucose levels. Mouse strains found to be susceptible, as indicated by the development of high persistent hyperglycemia by either of the above treatments, were retested using a lower single dose of 150 mg/kg or lower multiple doses of 30 mg/kg/day for 5 days. Strains found to be resistant, as indicated by low or insignificant elevation in blood glucose levels, were tested

TABLE 1
Hyperglycemic response in congenic resistant strains

Strain	Haplotype	*H-2* genotype K	IA	IE/C	D	Final level of plasma glucose (mg/dl)* Dose of STZ (mg/kg body wt × no. injections) 0	30 × 5	40 × 5	150 × 1	200 × 1
BALB.B	*b*	*b*	*b*	*b*	*b*	173 ± 6 (12)	ND†	276 ± 11 (23)	204 ± 8 (5)	525 ± 58 (18)
BALB.5R	*i*	*b*	*b*	*k/d*	*d*	166 ± 13 (3)	ND	280 ± 23 (12)	ND	599 ± 200 (2)
BALB.K	*k*	*k*	*k*	*k*	*k*	158 ± 7 (9)	ND	357 ± 32 (16)	234 ± 12 (5)	670 ± 65 (12)
BALB/cAn	*d*	*d*	*d*	*d*	*d*	163 ± 6 (19)	342 ± 55 (7)	411 ± 43 (25)	463 ± 55 (11)	583 ± 53 (19)
BALB.G	*g*	*d*	*d*	*d* / *b*		190 ± 4 (10)	ND	417 ± 52 (8)	370 ± 62 (5)	472 ± 5 (4)

Mice were injected with a single high dose or multiple low doses of STZ, and nonfasting plasma glucose levels were determined at semiweekly intervals for 4 wk as described in MATERIALS AND METHODS.
*Glucose level ± SD (no. of mice in group) as determined on day 28 after the first injection.
†Not determined.

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TABLE 2
Hyperglycemic response of inbred mice

H-2 Haplotype	Strain	Final level of plasma glucose (mg/dl)* Dose of STZ (mg/kg body wt × no. injections) 0	30 × 5	40 × 5	50 × 5	150 × 1	200 × 1	250 × 1
H-2^b	C57L/J	158 ± 6 (32)		256 ± 15 (15)	442 ± 67 (6)		988 ± 25 (9)	
	C57BL/6J	168 ± 3 (39)		291 ± 24 (14)	405 ± 77 (6)	179 ± 22 (3)	600 ± 57 (12)	
	C57BL/10SnJ	163 ± 8 (20)		534 ± 50 (9)			1045 ± 41 (8)	
H-2^k	C3H/An	119 ± 5 (5)		217 ± 14 (9)			489 ± 35 (8)	
	C3H/HeJ	169 ± 4 (36)	171 ± 7 (4)	377 ± 46 (13)			350 ± 62 (14)	732 ± 25 (5)
	AKR/J	181 ± 3 (44)	210 ± 13 (13)	362 ± 35 (20)			766 ± 35 (9)	
	CBA/J	188 ± 4 (40)		500 ± 31 (13)	654 ± 21 (6)		365 ± 20 (14)	487 ± 142 (2)
H-2^d	BALB/cAn	163 ± 6 (19)	342 ± 55 (7)	411 ± 43 (25)		463 ± 55 (11)	583 ± 53 (19)	
	C57BL/KsJ	206 ± 6 (34)	288 ± 27 (9)	576 ± 47 (5)			749 ± 19 (8)	
	DBA/2J	174 ± 4 (40)	443 ± 71 (5)	592 ± 20 (16)		467 ± 193 (4)	609 ± 29 (9)	
H-2^s	SJL	202 ± 5 (23)	226 ± 18 (5)	508 ± 83 (7)			730 ± 94 (8)	
H-2^a	A/J	164 ± 3 (51)	379 ± 58 (18)	497 ± 56 (21)		869 ± 68 (3)	(no survivors) (8)†	

See footnotes to Table 1.
*Glucose level ± SD (no. of mice in group) on day 28. Blank space denotes "not determined."
†Mice in this group all died of severe hyperglycemia before day 28.

for the second time with a single dose of 250 mg/kg and multiple doses of 50 mg/kg/day for 5 days.

The hyperglycemic response of the 5 congenic resistant strains of mice given the standard treatments is presented in Figure 1. All strains developed persistent hyperglycemia (range, 400–680 mg glucose/dl of plasma) within a few days after receiving the single high dose (200 mg/kg) (Figure 1A). However, there were significant interstrain differences in the hyperglycemic response when the mice were treated with the multiple low doses (40 mg/kg/day for 5 days) (Figure 1B). In Table 1, the known cross-over points within the *H-2* complex that distinguish each of the 5 congenic strains are indicated, together with the final level of hyperglycemia obtained after four different levels of STZ treatment. From these data, it appears that mice of the strains BALB.B (*H-2*^{b}) and BALB.5R (*H-2*^{i}) were the most resistant to the low-dose treatment, while mice of BALB.G (*H-2*^{g}) and BALB/cAn (*H-2*^{d}) background were the most susceptible. Since these congenic resistant strains are genetically similar, except for the known differences in the *H-2* region as indicated in Table 1, our observations suggest that factors associated with the *H-2* complex of genes have a strong influence on STZ sensitivity.

We next examined 12 inbred strains of mice for their relative susceptibility to STZ-induced hyperglycemia. The results are summarized in Table 2. All inbred strains became hyperglycemic (range, 490–990 mg/dl) when given the single high dose, with the exception of C3H/HeJ and CBA/J. Both of these latter strains, which are *H-2*^{k}, developed only a moderate hyperglycemia (350 ± 62 and 365 ± 20, respectively). To the multiple low-dose treatment, two strains, C3H/An and C57BL/6J, were relatively resistant and two

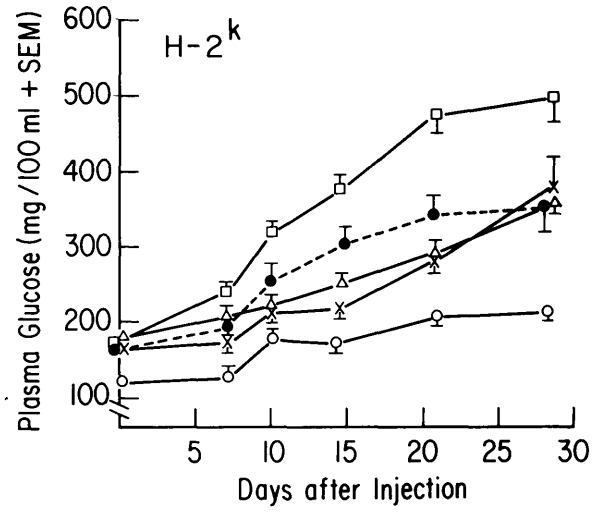

FIGURE 2. Influence of genetic factors not associated with the *H-2* region on hyperglycemia induction by STZ. Mice of different inbred strains sharing the same *H-2* haplotype (*H-2*^{k}) were injected with multiple low doses of STZ (40 mg/kg body wt/day for 5 days) on days 0–4, and examined for development of hyperglycemia as in Figure 1. —□—□— CBA/J; ---●---●--- BALB.K; —△—△— AKR/J; —X—X— C3H/HeJ; and —○—○— C3H/An.

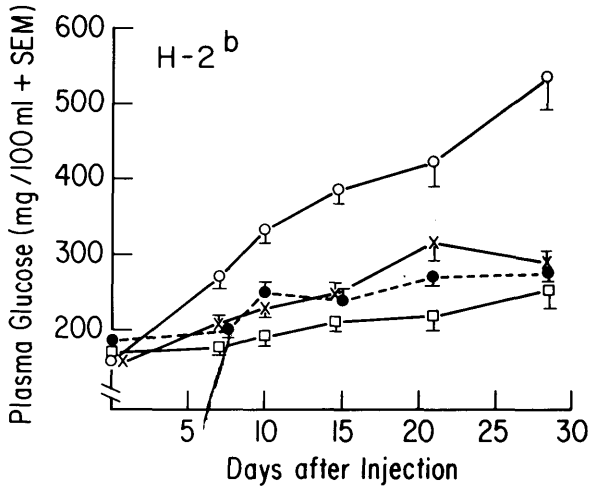

FIGURE 3. Influence of genetic factors not associated with the *H-2* region on hyperglycemia induction by STZ. Other conditions were as in Figure 2, except that mice sharing the *H-2*^{b} haplotypes were used. —○—○— C57BL/10SnJ; —X—X— C57BL/6J; ---●---●--- BALB.B; and —□—□— C57L/J.

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other strains, C3H/HeJ and AKR/J, were only moderately susceptible; all other strains were highly susceptible. It is apparent from Table 2 that a number of strains that share the same *H-2* haplotype may be quite different in their susceptibility to the hyperglycemic effect of multiple low doses of STZ. For instance, C57BL/6J and C57BL/10SnJ have an identical *H-2* haplotype (*b*), and C3H/An and CBA/J also share the same *H-2* haplotype (*k*), and yet these strains differed significantly in susceptibility.

The four strains with the *H-2*^{k} haplotype have been examined to demonstrate the intrahaplotype differences in STZ susceptibility after multiple low-dose injections. The results are presented in Figure 2. These strains (C3H/An, C3H/HeJ, AKR/J, and CBA/J) together represented a wide range of susceptibility; CBA/J was one of the most susceptible and C3H/An was the least susceptible among the 12 inbred strains examined in this study.

Similarly, with the *H-2*^{b} haplotype, C57BL/10SnJ was highly susceptible while C57L/J and C57BL/6J were less susceptible (Figure 3). In contrast, however, all three strains with the *H-2*^{d} haplotype (BALB/cAn, C57BL/KsJ, and DBA/2J) were essentially all susceptible (Table 2).

DISCUSSION

In these studies, we attempted to determine whether the resistance or susceptibility to STZ-induced diabetes in mice is influenced by genes associated with major histocompatibility complex and/or by determinants outside this complex. Earlier studies from several laboratories, including our own, have supported the view that diabetes induction by multiple low doses of STZ (but not by the high dose) may be mediated by an autoimmune mechanism requiring host T-cell functions.[3-7,10,14,15] If this view is correct, the host *H-2* type would be expected to have a strong influence on susceptibility or resistance to diabetes induction, since the major immune response genes are located within this gene complex.[8-11] It is widely recognized that the risk to type I diabetes in man is very significantly influenced by *HLA*-associated genes,[1,2] prompting the hypothesis that autoimmune processes also play an etiologic role in this disease.

Virus-induced leukemia in the mouse was the first disease to which susceptibility or resistance was shown to be influenced by the *H-2* complex.[16] The mechanism of this influence has since been shown to be primarily immunologic in nature; the capacity to generate a strong cytotoxic T-lymphocyte response to virus-derived cell surface antigens of syngeneic leukemia cells varies with the host's *H-2* type and correlates well with overall resistance to the disease.[17] However, any of a number of other murine genes can be involved in resistance to viral leukemia, depending on the mouse strain studied. In fact, possession of an *H-2* haplotype favoring resistance cannot, in the absence of at least one further resistance gene, confer more than a significant prolongation of the latency period of the disease after virus inoculation.[18]

Our results from the five congenic resistant strains examined in the present study show that mice that differ from each other only in their *H-2* region genes may differ significantly in their hyperglycemic response to multiple low-dose treatment with STZ. Thus, mice of the strain BALB.B (*H-2*^{b}) were quite resistant, while mice of the strains BALB/cAn (*H-2*^{d}) and BALB.G (*H-2*^{g}) were highly susceptible (Table 1). We therefore conclude that genes within the *H-2* complex indeed contribute significantly to the susceptibility. Moreover, our data appear to suggest that alleles on the left half of the *H-2* complex, the K and/or IA genes, have the major influence, since BALB.5R mice (haplotype *b b k/d d*) were much less susceptible compared with BALB.G mice (haplotype *d d d b*) (see Table 1). A definitive answer must await a more detailed analysis using additional recombinant and hybrid strains of mice.

On the other hand, our results from the 12 inbred strains indicate that *H-2*-associated genes cannot be the only determinants of susceptibility in this experimental disease. For example, C3H/An mice carrying the *H-2*^{k} haplotype were quite resistant, but CBA/J mice sharing the same *H-2*^{k} haplotype were highly susceptible. Likewise, C57BL/6J and C57BL/10SnJ mice have an identical *H-2* haplotype and yet show quite different hyperglycemic responses. These data demonstrate that genes outside the *H-2* region also have an influence on susceptibility or resistance.

Our observations are, in general, consistent with the conclusions of Rossini and colleagues,[8] whose study of 8 inbred strains showed high susceptibility for one strain of the *H-2*^{d} haplotype, intermediate susceptibility for an *H-2*^{b} strain, and low susceptibility for other strains representing *a, d,* and *k* haplotypes. Based on these results, they concluded that *H-2*-associated genes were not the major determining factors of susceptibility. Our observations with inbred strains differ from their results only in the degree of hyperglycemia that developed in some of the inbred strains of mice. For instance, C57BL/KsJ mice became severely hyperglycemic on day 10 after STZ treatment in the study of Rossini et al., while in our study these mice developed a frank hyperglycemia only after 21 days. Similar differences were seen also for A/J and C57BL/6J mice. These variations may have been due to environmental variations.[13,14] However, our results demonstrate further that the influence of *H-2*-associated genes become apparent only when strains of mice that differ only at the *H-2* region are used for comparison; Rossini et al. did not examine congenic strains in their study.

The results of the present study are partly in agreement with the reports of Kiesel and Kolb, who had found a modulating effect of the *H-2*-associated genes in diabetes inducted by STZ.[9,10] Our observations, however, differ from these authors in the identity of the *H-2* alleles correlated with high susceptibility. They reported that mice with the *k* haplotype are highly susceptible, and mice with the *b, d,* and *a* haplotypes were moderately so. Mice with the *s* haplotype (strain B10.S) were found to be resistant in their study. In contrast, our own results indicate that both the *k* and *d* haplotypes are associated with high susceptibility.

These considerations together lead us to the conclusion that susceptibility or resistance to progressive hyperglycemia, as induced by multiple low doses of STZ, is strongly influenced by at least two separate genes, at least one of which is located within the *H-2* complex and others outside the complex.

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