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The Influence of Genetic Background on the Susceptibility of Mice to Diabetes Induced by Alloxan and on Recovery from Alloxan Diabetes

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Summary. Alloxan was administered at dosages of 25 to 100 mg/kg to C57BL/6J (6J) and C57BL/KsJ (KsJ) mice to determine the dose dependence of alloxan-induced diabetes in these strains. KsJ mice were more susceptible to alloxan: the effective dose (ED) with respect to the likelihood of 50% of the treated mice becoming hyperglycaemic by 2 days was 42 mg/kg for KsJ mice, compared with 59 mg/kg for 6J mice ($P < 0.001$). The dose response curves for the two strains were parallel, and mice receiving equieffective alloxan dosages studied, which were approximately the ED_1, ED_{20}, ED_{80}, and ED_{99}, blood glucose levels at 2 days did not differ significantly between KsJ and 6J mice. Although the initial severity of hyperglycaemia and polydipsia was indistinguishable in KsJ and 6J mice administered the ED_{80} alloxan dosages, there were two major differences in the long term course of their diabetes. First, only 13 of 23 diabetic KsJ mice survived for three months following alloxan administration compared with all 23 diabetic 6J mice ($P < 0.001$). Second, 9 of 23 diabetic 6J mice gradually became normoglycaemic, compared with none of the diabetic KsJ mice ($P < 0.001$). These observations support an earlier hypothesis that these two strains of mice differ in their capacity to adapt to diabetogenic stimuli.

Key words: Alloxan, diabetes, mice, glucose tolerance, genetic.

Genetic background is believed to play a major role in determining individual susceptibility to diabetes and the severity and progression of the disease. Experimental diabetes in inbred strains of mice constitute model systems for examining such genetic background influences in detail. The susceptibility of mice to the development of diabetes due to alloxan [1], streptozotocin [2], or encephalomyocarditis virus [3] is known to vary among different inbred strains. However, study of the influence of genetic background on the progression of diabetes, after its onset, has been limited to a single model system developed by Coleman and colleagues [4, 5, 6]. In this model the non-allelic mutations, obese and diabetes-2J, which cause hyperphagia, were compared in C57BL/6J (6J) and C57BL/KsJ (KsJ) strains of mice. The two mutations produced similar syndromes in both mouse strains, while the two strains differed strikingly in their adaptations to either form of hyperphagia. In both strains, hyperphagia was associated with obesity and with early increases in blood sugar and insulin concentrations. In 6J mice, there followed sustained and profound pancreatic islet hypertrophy and hyperinsulinism with mild hyperglycaemia. By contrast, hyperphagia in KsJ mice produced transitory hyperinsulinism followed by islet degeneration and severe diabetes.

These findings suggested the hypothesis that the pancreatic islets of genetically normal 6J and KsJ mice differ in their capacity to undergo compensatory hypertrophy. In this report, we compare normal 6J and KsJ mice with respect to their susceptibility to the diabetogenic effect of alloxan and with respect to the progression or remission of diseases among mice administered an equieffective alloxan dosage.

Materials and Methods

Male C57BL/6J (6J) and C57BL/KsJ (KsJ) mice were obtained from the Jackson Laboratory, Bar Harbor, Maine, and were fed Purina Laboratory Animal Chow ad lib. At ten-thirteen weeks of age (20–22g) the two strains of mice were injected in a lateral tail vein with 100 µl of a solution containing variable amounts of alloxan, freshly prepared in 10 mmol/l citrate in 0.154 mmol/l NaCl,

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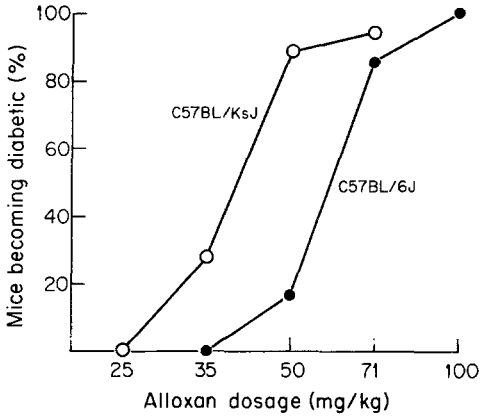

Fig. 1. Dose dependence of alloxan-induced diabetes in fed C57BL/6J and C57BL/KsJ mice. They received the indicated alloxan dosages and blood glucose levels were determined after two days. Diabetic mice were identified as described in Materials and Methods, generally on the basis of two day blood glucose levels exceeding 250 mg/dl. The number of mice receiving each alloxan dosage is listed in Table 2

pH 4.4 [7]. All injected mice survived for at least two weeks except a single mouse which died two days after alloxan administration. The data for this mouse was omitted from all calculations.

Blood sampling (25 to 100 µl) was performed by orbital puncture using heparinized capillary tubes. All mice were bled at 2, 30 (27 to 33), 60 (55 to 65) and 90 (80 to 100) days after alloxan administration. In some experiments, mice were also bled at 4, 7, 14, or 44 days to confirm trends. All bleedings and injections were performed at the same time of the day (2:00 pm).

Samples for whole blood glucose determinations were routinely frozen before analysis by one of two glucose oxidase methods (glucose analysis kit, Sigma or glucose analyzer, model 23A, Yellow Springs Instruments). The correlation between the two methods was 96. In some experiments, the blood glucose concentration was calculated from the haematocrit and plasma glucose concentration of fresh specimens.

Mice were generally considered diabetic if their two day blood glucose level exceeded 250 mg/dl. There were six mice for which this criterion was unsatisfactory. Four mice had two day blood glucose levels between 175 and 250 mg/dl and several subsequent levels above 350 mg/dl; these mice were considered diabetic. In addition, two mice whose two blood glucose levels were approximately 300 mg/dl were normoglycaemic within a week of alloxan administration and were not considered diabetic.

Mean blood glucose concentrations were compared using Student's t-test. Proportions of mice responding to a single condition are compared using the χ^2value of the two-by-two table of responders and non-responders. The dose dependences of 6J and KsJ mice becoming diabetic after different alloxan dosages were compared by the simplified method of Litchfield and Wilcoxon [8].

Results

The proportions of mice of the C57BL/6J and C57BL/KsJ strains which became diabetic following administration of different alloxan dosages are shown in Figure 1. The two strains differed significantly in the number of mice which became diabetic among those receiving 50 mg/kg alloxan ($P < 0.001$).

Table 1. Calculated parameters of dose dependence of alloxan-induced diabetes

	C57BL/KsJ	C57BL/6J
Ed_{50}, mg/kg alloxan	41.7 (37.8–46.1)[a]	59.4 (54.2–65.1)
Slope of dose dependence	1.27 (1.19–1.36)	1.23 (1.17–1.30)

[a] 95% confidence intervals are in parentheses

Table 2. Blood glucose levels two days after alloxan administration in KsJ and 6J mice

Relative dose	Alloxan dose mg/kg	C57BL/KsJ n	Blood glucose mg/dl	Alloxan dose mg/kg	C57BL/6J n	Blood glucose mg/dl
ED_1	25	8	132 ± 11	35	8	125 ± 8
ED_{20}	35	18	202 ± 28	50	12	145 ± 21
ED_{80}	50	27	373 ± 24	71	28	373 ± 22
ED_{99}	71	19	525 ± 37	100	13	437 ± 24

Equieffective alloxan dosages with respect to the likelihood of treated mice developing diabetes were identified using the data from Table 1. Blood glucose levels of all treated mice, whether diabetic or not, are included. At none of the equieffective dosages did C57/BL/KsJ and C57/6J mice differ significantly in mean blood glucose ($P > 0.05$). Results are shown as mean ± SEM where appropriate

The dose dependence of alloxan-induced diabetes in these strains was compared over the full range of tested dosages using the method of Litchfield and Wilcoxon [8]. These calculations yield ED_{50} doses and slopes listed in Table 1. The slopes of dose dependence for 6J and KsJ mice did not differ significantly, indicating that the dose response curves in Figure 1 are parallel and can be compared. The ED_{50} of alloxan diabetogenesis among 6J mice was 59.5 mg/kg, compared with ED_{50} for KsJ mice of 41.5 mg/kg ($P < 0.001$), giving a mean susceptibility ratio (ratio of ED_{50}'s) of 1.43 with 95% limits of 1.23 and 1.65.

It was calculated that 6J mice administered 71 mg/kg alloxan received an equieffective dosage to KsJ mice administered 50 mg/kg, which was approximately the ED_{80} with respect to the likelihood of treated mice becoming diabetic. This was found to be the alloxan dosage at which the largest proportion of injected mice became diabetic and survived at least two months.

The initial severity of diabetes is indicated by the blood glucose concentrations 2 days after alloxan administration. The blood glucose levels of 6J and

KsJ mice treated with different equieffective alloxan dosages were compared (Table 2). At the alloxan dosage of the ED_{80} the degree of hyperglycaemia at two days was the same for injected 6J and KsJ mice; the mean blood glucose was 373 ± 24 mg/dl for KsJ mice and the same, 373 ± 22 mg/dl, for the 6J mice. At two of the other dosages compared, the ED_{20} and the ED_{99}, the mean two day blood glucose levels were higher for KsJ mice than for 6J mice, but the differences were not significant ($P > 0.05$). At the ED_1 dosage both KsJ and 6J mice were normoglycaemic at two days.

In other experiments six mice of each strain received three doses of alloxan, each equal to the ED_1, at two week intervals. None of these mice became hyperglycaemic, although the total amount of alloxan they received would have exceeded the ED_{99} if it were administered as a single injection.

The mean degrees of polydipsia, a time-averaged indicator of the severity for one group of 6J and KsJ mice administered the ED_{80} of alloxan, are shown in Figure 2. Both strains of mice showed similar degrees of polydipsia during the period from two to seven days following alloxan administration.

The survival of alloxan-treated, diabetic 6J and KsJ mice is compared in Figure 3. Diabetic mice of the two strains, which had received alloxan at the ED_{80} dosage, differed in their survival. All 6J mice survived for at least three months. Although all diabetic KsJ mice survived the first month, five died during the second month ($P < 0.02$) compared with 6J mice, and five more died during the third month ($P < 0.001$). The two day mean blood glucose level for diabetic 6J mice which recovered subsequently was 373 mg/dl and is slightly lower than the mean blood glucose level for diabetic 6J mice which did not recover ($P < 0.05$). During the subsequent three months, the mean blood glucose levels of recovering diabetic 6J mice gradually returned to normal. The mean blood glucose level of these mice one month after alloxan administration was significantly less than at two days ($P < 0.001$) and was significantly greater than the normoglycaemic levels attained by three months ($P < 0.05$).

Among KsJ diabetic mice blood glucose levels increased significantly from two days to one month after alloxan administration ($P < 0.01$) and did not vary significantly thereafter. This increase in mean blood glucose level correlated with an increase in water consumption at the same time (Figure 2). By contrast, water consumption among 6J mice injected with an equieffective alloxan dosage at the same time was unchanged from two to thirty days following treatment (Figure 2).

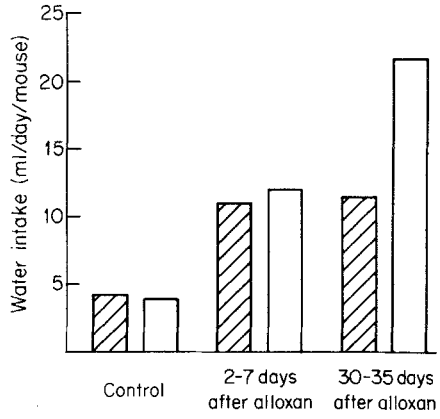

Fig. 2. Polydipsia in alloxan-induced diabetes. Daily water consumption for cages of six to ten mice was measured for five consecutive days. The day-to-day variation for each group was small and is not shown (SEM < 5% in all cases). Diabetic mice had received the ED_{80} dosage of alloxan. ▨ = C57BL/6J, □ = C57BL/KsJ

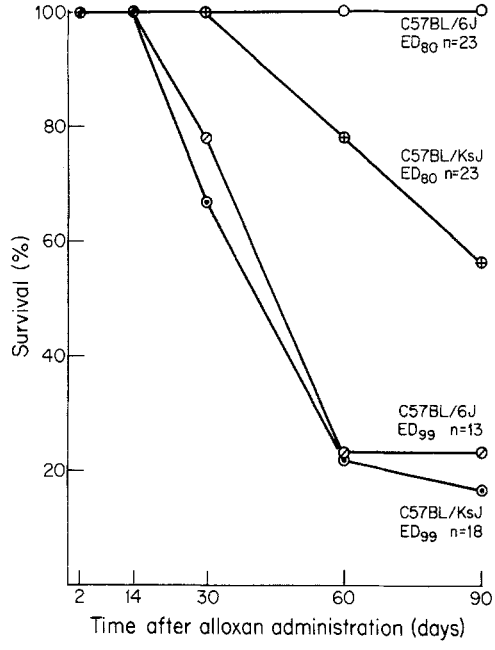

Fig. 3. Survival of diabetic mice after ED_{80} and ED_{99} doses of alloxan

Discussion and Conclusion

The data presented in this paper indicate that alloxan is a more potent diabetogenic agent in KsJ than in 6J mice. The fact that mouse strains vary in their susceptibility to alloxan was first documented by Martinez [1]. Several substances can inhibit or potentiate the actions of alloxan in vivo, including glutathione and ascorbic acid [7]. Strain variations in the levels of

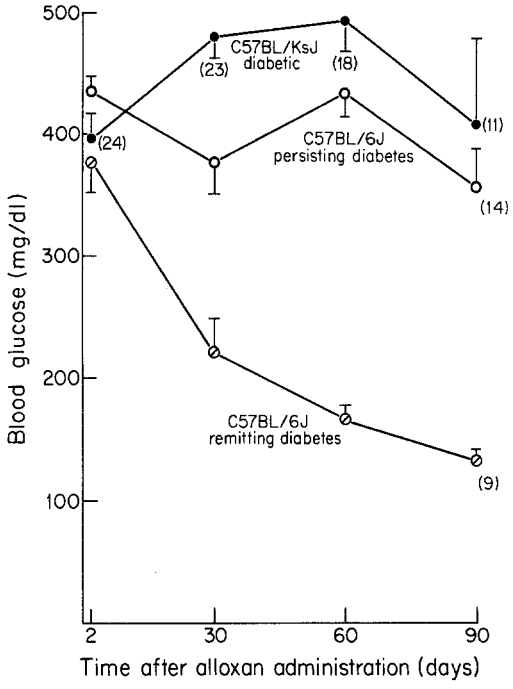

Fig. 4. The timecourse of hyperglycaemia in diabetic mice which received the ED_{80} dosage of alloxan. The number of mice alive at the designated time points is shown in parenthesis; where a number is given at 90 days only, no deaths were observed during the experiment

such substances could account for observed differences in the potency of alloxan. Rossini and colleagues [2] have recently reported that KsJ mice are more susceptible than 6J mice to repeated subdiabetogenic streptozotocin dosages. This is believed to occur because streptozotocin elicits an inflammatory reaction in the islets of KsJ but not 6J mice. However, repeated subdiabetogenic administration of alloxan did not produce a similar insulitis [9]. Therefore, the different susceptibilities of the two strains to repeated low streptozotocin dosages and to a single alloxan dosage probably reflect separate phenomena.

The blood glucose level at 2 days was selected as an indication of the initial severity of alloxan diabetes based on the description by Rerup and Lundquist [10] of the early events following alloxan administration to fed, female NMRI mice. Three phases of developing diabetes were identified using a dosage of alloxan which produced sustained diabetes in over 95% of 1200 treated mice. During the first 2 hours there is hyperglycaemia associated with hepatic glycogenolysis and hypoinsulinaemia. There follows a phase of hyperinsulinaemia and hypoglycaemia lasting 4–10 h. The third phase of sustained hyperglycaemia is evident at 24 h and is associated with low or undetectable insulin levels. Diabetes is fully manifest at 48 h in two respects: all treated mice who become diabetic do so within 2 days and mean blood glucose concentrations are the same at 2 days and at 26 days. A similar sequence of blood glucose alterations was observed in Swiss Webster mice [11]. Within 2 days all (of 150) treated mice were diabetic and maximal blood glucose levels had been reached.

Studies of pancreatic histology following alloxan administration in other mouse strains and other species support the concept that beta cell necrosis, with release of stored insulin, is responsible for the phase of hyperinsulinaemia and hypoglycaemia [7, 12]. The ensuing sustained hyperglycaemia is attributed to a decreased remaining beta cell mass, which is unable to release sufficient insulin for glucose homeostasis.

In this study we identified equieffective alloxan dosages in 6J and KsJ mice with respect to the likelihood of treated mice becoming diabetic, and we found that mice administered the ED_{80} alloxan dosage develop equally severe initial (2d) hyperglycaemia. Although alloxan at these dosages represents a comparable diabetogenic stimulus in these two strains, there are important differences in the progression of diabetes in 6J and KsJ mice. In KsJ mice hyperglycaemia becomes progressively more severe, increasing significantly during the first month. Although mean blood glucose levels did not increase further, several mice died during each of the subsequent 2 months. This progression in the severity of alloxan diabetes in KsJ mice is reminiscent of the syndromes produced in KsJ mice by both the obese and diabetes mutations, in which premature death is typical [5].

Two distinct patterns of alloxan diabetes were observed in 6J mice administered the ED_{80} alloxan dosage. In 9 of 23 diabetic mice there was spontaneous remission of diabetes, with progressively less severe hyperglycaemia at 1 and 2 months and normoglycaemia at 3 months. In the remaining 14 mice diabetes persisted without becoming more or less severe during the subsequent 3 months. All diabetic 6J mice survived 3 months. It is not clear why some 6J mice should have recovered from diabetes spontaneously, while other, genetically identical mice showed not even a tendency towards less severe hyperglycemia during the 3 months. The fact that recovering mice had slightly lower 2 day blood glucose levels raises the possibility that these mice had slightly less severe diabetes initially.

The spontaneous remission of diabetes observed in several 6J mice administered the ED_{80} alloxan dosage is similar to the pattern of alloxan diabetes described by Bunnag et al [11] in Swiss Webster mice. In that study a higher alloxan dosage was

administered, causing diabetes in all mice and death in several. However, surviving diabetic mice became gradually less hyperglycaemic until they were all normoglycaemic by 2–3 months. The return to normoglycaemia in that study correlated with an increase in the number of pancreatic islets in diabetic mice, from 17% of saline-treated controls at 1 week after alloxan administration, to 62% at 3 months. This increase in the number of islets was associated with increased thymidine uptake in pancreatic duct and insular cells, especially during the 2 to 8 weeks following alloxan administration. Since the remission from alloxan diabetes in 6J mice which we report here is similar in its time course, occurring gradually during 3 months, we believe that a similar increase in pancreatic islet mass and function may be the underlying mechanism for remission in 6J mice. Our working model is that remission of alloxan-induced diabetes in 6J mice reflects compensatory hypertrophy or hyperplasia of those pancreatic beta cells which survive alloxan administration, and are then subjected to an increased insulin demand relative to the number of insulin secreting cells.

Coleman and Hummel [5] have concluded that 6J and KsJ mice, which are homozygous for mutations causing hyperphagia, differ in their capacity to expand insulin supply. Mice of the 6J strain with either the diabetes-2J or obese mutations appear to be able to expand insulin supply indefinitely, while KsJ mice with these mutations are unable to adapt to increasing demand for insulin. The observation reported in this study that 6J and KsJ mice differ in their ability to recover from alloxan-induced diabetes supports the hypothesis of Coleman and Hummel that the capacity to expand insulin supply in normal 6J and KsJ mice is genetically determined.

Pancreatic islet adaptations may play a major role in the pathogenesis of human adult onset diabetes. Obesity and several endocrine disturbances, which predispose to human diabetes, may be stimuli of compensatory hypertrophy in the islets of the normal individual. Diabetes would then become latent or manifest when such an adaptation is insufficient. A variety of factors might influence the capacity of an individual's islets to adapt, including genetic background, nutrition, toxin and drug exposure and prior exposure to stresses. The identification and evaluation of conditions conducive to successful pancreatic beta cell adaptations requires in vivo animal models. In this study, we report the role of one such factor, genetic background, in the adaptation of mice to diabetes induced by alloxan.

References

1. Martinez, C., Grande, F., Bittner, J. J.: Alloxan diabetes in different strains of mice. Proc. Soc. Exp. Biol. Med. **87**, 236–237 (1954)
2. Rossini, A. A., Appel, M. C., Williams, R., Like, A. A.: Genetic influence of the streptozotocin-induced insulitis and hyperglycemia. Diabetes **26**, 916–920 (1977)
3. Boucher, D. W., Hayaski, K., Rosenthal, J., Notkins, A. L.: Virus-induced diabetes mellitus. III. Influence of sex and strain of the host. J. Infect. Dis. **131**, 462–466 (1975)
4. Hummel, K. P., Coleman, D. L., Lane, P. W.: The influence of genetic background on expression of mutations at the diabetes locus in the mouse. I. C57BL/KsJ and C57BL/6J strains. Biochem. Gen. **7**, 1–13 (1972)
5. Coleman, D. L., Hummel, K. P.: The influence of genetic background on the expression of the obese (ob) gene in the mouse. Diabetologia **9**, 287–293 (1973)
6. Coleman, D. L., Hummel, K. P.: The influence of genetic background on the expression of mutations at the diabetes locus in the mouse. II. Studies on background modifiers. Isr. J. Med. Sci. **11**, 708–813 (1975)
7. Rerup, C. C.: Drugs producing diabetes through damage of the insulin secreting cells. Pharmacol. Rev. **22**, 485–518 (1970)
8. Litchfield, J. T., Jr., Wilcoxon, F.: A simplified method of evaluating dise-effect experiments. J. Pharmacol. Exp. Ther. **96**, 99–113 (1949)
9. Rossini, A. A., Like, A. A., Chick, W. L., Appel, M. C., Cahill, G. F.: Studies of streptozotocin-induced insulitis and diabetes. Proc. Natl. Acad. Sci. USA **74**, 2485–2489 (1977)
10. Rerup, C., Lundquist, I.: Blood glucose level in mice. 2. A quantitative study of alloxan diabetes. Acta Endocrinol. (Kbh.) **54**, 514–526 (1967)
11. Bunnage, S. C., Walker, N. E., Bunnage, S.: Effect of alloxan on the mouse pancreas during and after recovery from diabetes. Diabetes **16**, 83–89 (1967)
12. Patent, G. J., Alfert, M.: Histological changes in the pancreatic islets of alloxan-treated mice, with comments on β-cell regeneration. Acta Anat. (Basel) **66**, 504–519 (1967)

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