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Multiple low-dose streptozotocin-induced hyperglycemia and insulitis in C57BL mice: Influence of inbred background, sex, and thymus

(diabetes/inbred mice/*nude* mice)

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ABSTRACT **Insulin-dependent diabetes induced in susceptible strains of mice by multiple, low-dose streptozotocin treatment has been proposed to entail a thymus-dependent, autoimmune destruction of beta cells. In this study, thymectomized and genetically athymic mice have been tested for susceptibility to streptozotocin. Thymectomy was performed on newborn (day 1) to 3-day-old C57BL/KsJ mice. At 8 wk of age, thymectomized and sham-operated mice of both sexes were tested for susceptibility to diabetes induction by multiple, low-dose streptozotocin treatment (35 mg/kg of body weight per day for 6 consecutive days). Thymectomy failed to block susceptibility of males to induction of severe hyperglycemia. Beta cell necrosis and inflammatory cell infiltrates (insulitis) were consistent histopathological features. In general, females—both thymus-intact and thymectomized—were less susceptible than males to streptozotocin-induced hyperglycemia, and females exhibited an equally severe insulitis by experimental day 14; thus, the detection of an underlying insulitis did not predict the development of a more severe hyperglycemia because most streptozotocin-treated females at experimental day 35 continued to show only a modest hyperglycemia (about 200 mg/dl) compared to males (>400 mg/dl). That streptozotocin-induced hyperglycemia could occur in the absence of an intact thymus was further demonstrated in genetically athymic C57BL/6J NIcrOu *nu/nu* males and thymus-intact +/? littermate controls. C57BL/6J mice were resistant to streptozotocin-induced insulitis. This study shows that the presence of insulitis does not necessarily presage onset of severe hyperglycemia (e.g., C57BL/KsJ females), and conversely, the presence of severe hyperglycemia after low-dose streptozotocin treatment is not necessarily diagnostic of an underlying insulitis (e.g., C57BL/6J +/? and *nu/nu* males). These data stress the need for caution in the interpretation of studies of streptozotocin-insulitis sensitivities of *nude* mice.**

Insulitis (the presence of a mononuclear cell infiltrate within the pancreatic islets) has been one of the histopathological features associated with acute-onset type I insulin-dependent ("juvenile") diabetes (1). Insulitis has also been associated with the spontaneous development of acute-onset insulin-dependent diabetes in BB/W rats (2) and NOD mice (3). An experimental system offering a means to assess the etiopathologic significance of insulitis in induction of diabetes has been developed by Like and Rossini (4–6). They administered the diabetogenic antibiotic streptozotocin (SZ) to randomly bred CD-1 male mice daily for 5 days at a dose of 40 mg/kg of body weight. Although each daily dose was subdiabetogenic, the multiple low-dose regimen produced hyperglycemia and insulitis within 5–6 days after the last SZ injection. Administration together with glucose analogs—such as 3-O-methylglucose—that presumably compete with the glucopyranose ring of SZ for binding to, or transport into, beta cells could transiently protect mice from multiple low-dose SZ diabetes. However, complete protection could be produced only by SZ administration together with glucose analogs plus chronic administration (up to 5 wk) of antilymphocyte serum (5).

These studies suggested two components in pathogenesis: a direct toxic effect of SZ on beta cells and a delayed inflammatory reaction against damaged beta cells. The finding of intracellular retrovirus induction within SZ-damaged beta cells several days prior to the insulitis led to the proposal that the inflammatory reaction might entail cell-mediated killing of beta cells that express a SZ-induced neoantigen, possibly related to the retrovirus induction (4). The possibility that this SZ-insulitis model in mice might have an autoimmune component is of considerable interest inasmuch as autoimmune-like pathogenetic mechanisms have been proposed to be associated with the development of type I insulin-dependent diabetes in man (7).

The question of whether the inflammatory cells associated with SZ-induced insulitis are effectors of beta cell cytolysis or whether they represent a secondary, systemic response to beta cell necrosis induced directly by SZ has not been satisfactorily resolved. An organism's ability to mount humoral and cell-mediated immune responses has been associated with normal thymus gland maturation. Accordingly, athymic *nu/nu* (*nude*) mice have been employed to assess the role of thymus-dependent immunity in SZ-insulitis induction. Buschard and Rygaard (8) found both BALB/cBom normal and *nu/nu* mice to be sensitive to the hyperglycemic action of a 5-day regimen of SZ (40 mg/kg). However, the athymic mice exhibited a lower hyperglycemia than did euthymic mice at experimental day 22. No severe insulitis was induced in either group (8). In contrast, Paik *et al.* (9), in repeating this study, found their BALB/cBom *nu/nu* mice to be completely insensitive to the hyperglycemic action of the multiple low-dose SZ regimen. By reconstituting the *nu/nu* mice with a thymus, sensitivity to SZ could be restored, but the induced hyperglycemia was still lower than that of unreconstituted *nu/nu* mice previously observed (8). Induction of abnormal glucose tolerance, but not of fasting hyperglycemia, could be achieved after injection of splenic lymphocytes from SZ-diabetic euthymic littermates. Prior treatment of these splenic lymphocytes to remove T cells, but not B cells, prevented this passive transfer of glucose intolerance (9). These results led Paik *et al.* (9) to conclude that thymus-dependent functions played an obligatory etiologic role in the low-dose SZ model. However, these authors provided no data to document the presence or absence of insulitis in their mice. Indeed, it had

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Abbreviations: SZ, streptozotocin; ShTx, sham-thymectomized; Tx, thymectomized.

previously been shown that BALB/cJ mice were resistant to induction of insulitis, severe hyperglycemia, and beta-cell retrovirus expression by the low-dose SZ protocol (10); of eight inbred strains examined, only the C57BL/KsJ mice exhibited this triad of responses (10).

In the present study, the effect of neonatal and postnatal thymectomy on the subsequent susceptibility of the C57BL/KsJ mice to low-dose SZ-induced insulitis and diabetes was examined. Furthermore, the question of an intrinsic resistance in genetically athymic mice to the hyperglycemic action of multiple low-dose SZ injections was reexamined by using an available stock of *nu/nu* mice on the C57BL/6J inbred background. C57BL/6J mice are closely related to C57BL/KsJ mice and, although resistant to SZ-induced insulitis, they are more sensitive to the hyperglycemic action of SZ than are BALB/cJ mice (10).

MATERIALS AND METHODS

C57BL/6J NIcrOu male *nu/nu* mice and normal (+/?) littermates were generously provided by H. C. Outzen of The Jackson Laboratory. These mice were maintained in a conventional colony in a limited-access facility that was supplied with filtered air under positive pressure. Mice were caged in double-pen, polycarbonate boxes that contained sterilized white pine shaving bedding; the boxes were covered by Lexon filter bonnets. The diet (OG 911A, Emory Morse, Old Guilford, CT) was pasteurized and the water was acidified and fortified with menadione sodium bisulfite (0.04 mg/ml). The mice were free of ecto- and endoparasites, mycoplasmas, common bacteria, and viral pathogens, with the exception of a sporadic appearance of murine hepatitis virus.

The C57BL/KsJ mice were produced in a research colony as described (11). The breeders were heterozygous for the genes misty (*m*) and diabetes (*db*) in coupling; only the black, lean (++/?) progeny were used in this study. Newborn litters (designated day 1 mice), as well as 2- and 3-day-old pups, were anesthetized by cooling on ice and were surgically thymectomized. After both lobes of the thymus were teased out with cotton swabs, the chest cavity was examined under a dissecting microscope to confirm completeness of gland removal. Sham-thymectomized (ShTx) mice were subjected to the same surgical manipulations as the thymectomized (Tx) mice except for thymus removal. After surgery the pups were kept under a heat lamp for 4 hr to ensure complete hemostasis before they were returned to the mother. Although postoperative mortality was <5%, cannibalism by the dam was a serious problem. Coating the backs of the pups with female urine prior to their return to the mother proved to be the most effective remedy.

At 8 wk, the lean, black ++/? mice (both males and females) were weighed and blood glucose in fed mice was determined by a glucose oxidase method (12). These mice were then injected with either citrate buffer (pH 4.2) or SZ (lot 60, 140-1, a generous gift from UpJohn) within 5 min after the SZ was dissolved in the citrate buffer. The multiple low-dose treatment of Like and Rossini (4) was employed with the modification that 35 mg of SZ per kg of body weight was injected intraperitoneally in a 0.1-ml volume daily for 6 days. This treatment was compared with the Like-Rossini protocol and the modified regimen was found to produce a similar delayed development of severe hyperglycemia. The advantage of the modified treatment was a more reproducible induction of hyperglycemia accompanied by insulitis. Blood glucose levels were determined at the intervals shown in the text. Insulitis induction was assessed by sacrificing mice 8 days after the last SZ injection (experimental day 14). At sacrifice, the mediastinal region of Tx mice was examined visually for the presence of thymic rudiments. The pancreas was fixed in Bouin's fluid. Ovaries from Tx and ShTx females were also fixed for histological determination of dysgenesis.

The severity of insulitis was scored in hematoxylin/eosin-stained 5-μm pancreatic sections; a scale from 0 to 4 (described in Fig. 4 legend) was employed. Each pancreas was sampled at five intervals along its length, and each sampling point was separated by at least 100 μm from the previous one. Five coded slides from each pancreas were analyzed; a minimum of 20–30 islets no smaller than 50 μm in diameter was scored for insulitis. An additional slide cut from each pancreas was stained with aldehyde fuchsin to determine the number of granulated beta cells per islet.

Statistical analyses were performed by using a paired-difference Student's *t* test where applicable. A multivariate analysis of variance was used to test for significant main effects of sex on blood glucose level over time. Significance was assumed at $P \leq 0.05$.

RESULTS

SZ Susceptibility of Euthymic and Athymic Mice. Data presented in Fig. 1 show sensitivity of BL/Ks and BL/6 mice to the hyperglycemic action of multiple, subdiabetogenic SZ treatments. BL/Ks males developed a severe, sustained hyperglycemia. In contrast, most, but not all, females of the same age were relatively resistant to induction of severe hyperglycemia; only 4 of 19 females developed a fed blood glucose level above 300 mg/dl at experimental day 14, and at experimental day 35, mean blood glucose in females was much lower ($\Delta > 200$ mg/dl) than the mean for males. This sex difference was highly significant ($P < 0.001$) at all time intervals after day 0. No mice receiving six injections of citrate buffer only (vehicle control) developed hyperglycemia (data not shown).

The sensitivity of BL/6 euthymic (+/?) and athymic (*nu/nu*) male mice to SZ-induced hyperglycemia is also shown in Fig. 1. The mean body weight of the six +/? males at 8 wk was 23.8 ± 0.6 g (SEM), whereas that of the six *nu/nu* males was 20.6 ± 0.4 g. Eight days after the last SZ injection (experimental day 14), both groups exhibited comparable, sustained hyperglycemia. Although initially lower than that produced in BL/Ks males, this hyperglycemia was higher than that produced in BL/Ks females at experimental day 14. However, blood glucose levels in BL/6 *nu/nu* males continued to increase and by day 35 attained a mean value almost identical to that seen in BL/Ks males at the same time, whereas the +/? mean glucose level was about 20% lower.

Effect of Thymectomy. Early thymectomy of BL/Ks mice resulted in very high preweaning mortality, with 48/128 (38%) surviving thymectomy between 1 and 3 days. Mortality was lower in the ShTx mice, with 50/66 (76%) surviving; these mice appeared to be healthy and exhibited a normal range of body weight and blood glucose values at 8 wk. Visual inspection of the mediastinal cavity of Tx mice at autopsy did not reveal the presence of residual thymic tissue. The ratio of surviving males to females was 2:1. Data in Fig. 2 show that thymectomy did not alter the susceptibility of BL/Ks males to multiple, low-dose SZ-induced hyperglycemia. SZ induced an almost uniform and highly significant ($P < 0.001$) elevation in mean blood glucose of all groups of males either Tx or ShTx between birth and postpartum day 3. There were no significant differences in blood glucose levels between SZ-treated males and their ShTx controls—mean blood glucose values on day 14 of diabetes induction ranged between 268 ± 25 mg/dl (day 1 Tx) and 325 ± 50 mg/dl (day 3 ShTx). Female susceptibility, notably lower in thymus-intact females than in males, was more variable than in males after thymectomy (Fig. 2). An insufficient number of 1-day Tx females was available for analysis; 1-day ShTx, as well

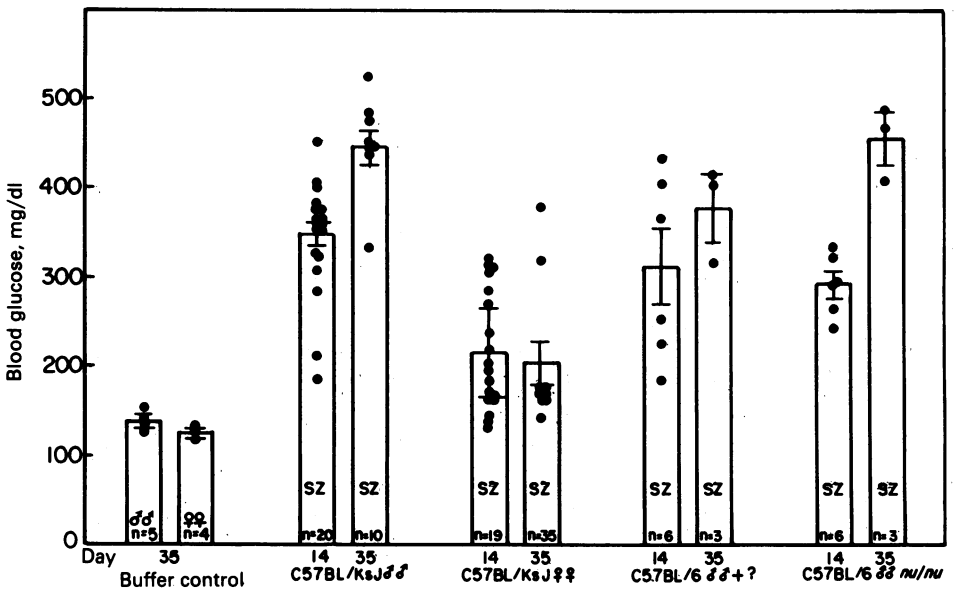

FIG. 1. Induction of hyperglycemia by multiple low-dose SZ administered during experimental days 1–6. Blood glucose values (●) of individual mice of the sex and genotypes shown are plotted with histograms of the mean ± SEM of the treatment group studied on experimental days 14 and 35. Buffer-injected BL/Ks and BL/6 mice gave almost identical blood glucose values between 125 and 150 mg/dl; only values for BL/Ks controls (far left) are shown. Significant differences ($P \leq 0.01$) at experimental days 14 and 35 were found in comparing all SZ-treated groups to the appropriate sex- and genotype-matched controls. Sex (maleness) in BL/Ks mice had a significant effect ($P < 0.001$) on hyperglycemia whereas thymus state in BL/6 male mice did not.

as 3-day Tx females, attained "male" levels of hyperglycemia at experimental day 14. A "female-like" modest elevation in blood glucose to approximately 220 mg/dl was exhibited by the 2- and 3-day ShTx females, whereas the 2-day Tx females failed to develop an elevated mean blood glucose level.

Insulitis Induction. Histological examination of the pancreases of all citrate buffer-treated control mice revealed intact islets showing no evidence of degenerative changes or of insulitis (Fig. 3*a*). An occasional focal inflammation of the stromal areas of the exocrine pancreas was the only lesion noted in this group. On the contrary, severe insulitis was observed on experimental day 14 in most of the thymus-intact, ShTx, and Tx BL/Ks mice of both sexes (Fig. 3 *b–d*). Table 1 summarizes the high frequency of severe insulitis that was detected upon autopsy at experimental day 14 of the Tx and ShTx mice whose blood glucose levels were shown in Fig. 2.

Fig. 4 presents a frequency distribution of the severity of insulitis in islets of some of the treatment groups in Figs. 1 and 2. Fig. 4*A* shows that in pancreases from five citrate buffer-

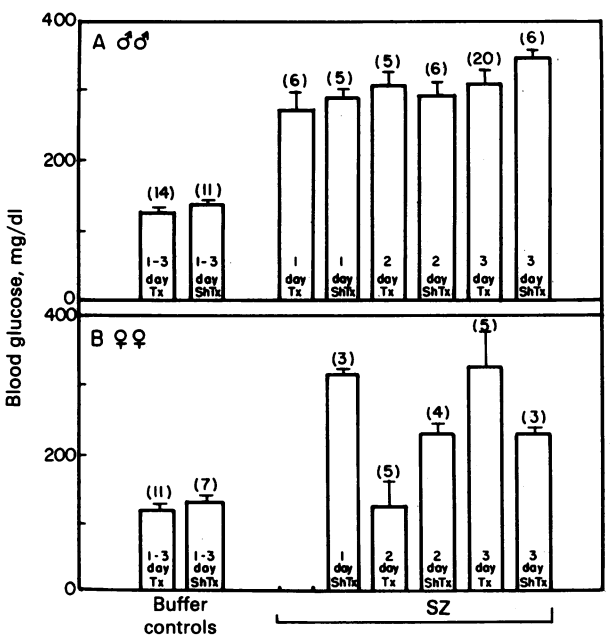

FIG. 2. Effect of neonatal (1-day) and postnatal (2- and 3-day) thymectomy (Tx) and sham-thymectomy (ShTx) on susceptibility of BL/Ks mice to hyperglycemia induction. Blood glucose was determined on experimental day 13 (7 days after last SZ injection). All groups of males (*A*) receiving SZ were significantly ($P \leq 0.001$) hyperglycemic compared to the appropriate buffer controls. All groups of females (*B*) receiving SZ with the exception of the 2-day Tx group exhibited significantly elevated ($P \leq 0.01$) blood glucose levels. Values are means with SEM. Number of mice per group is shown in parentheses.

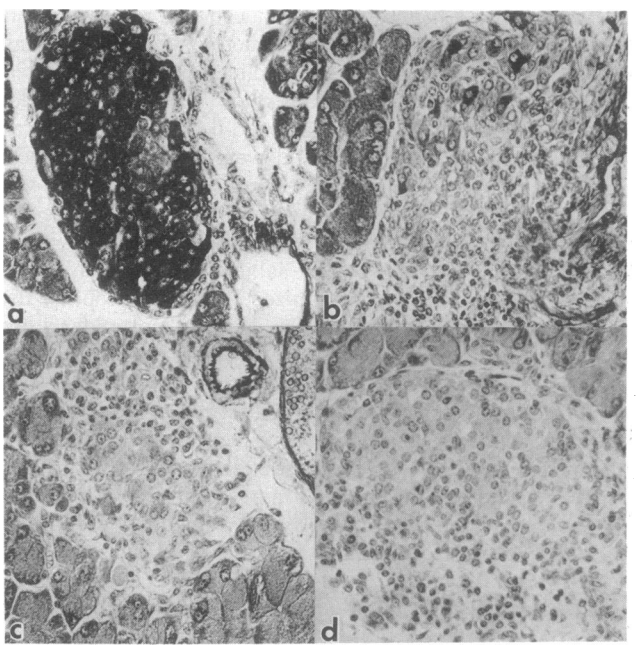

FIG. 3. Pancreatic islets of BL/Ks mice sacrificed 8 days after the last SZ injection. (*a*) Islet of a male receiving citrate buffer only. Numerous darkly staining (granulated) beta cells are present. (Aldehyde fuchsin; ×400.) (*b*) Thymus-intact female treated with SZ. Only a few granulated beta cells are seen; a heavy lymphocytic cell infiltrate permeates the interior and periphery of the islet. (Aldehyde fuchsin; ×400.) (*c*) Degenerate aldehyde fuchsin-negative islet observed in a neonatally Tx male. After SZ treatment, islets in this group are very small and degenerate and show varying degrees of residual insulitis. A heavy lymphocytic infiltrate is apparent in the islet depicted. (Aldehyde fuchsin; ×400.) (*d*) Islet from a neonatally ShTx male, showing the presence of a lymphoid cell infiltrate after SZ treatment. (Hematoxylin/eosin; ×400.)

Table 1. Inability of thymectomy or sham-thymectomy to block SZ-induced insulitis in BL/Ks mice

Treatment at 8 wk	Ratio, mice with severe insulitis/total					
	Day 1		Day 2		Day 3	
	ShTx	Tx	ShTx	Tx	ShTx	Tx
Buffer						
♂♂	0/4	0/4	0/5	0/2	0/2	0/7
♀♀	0/3	0/3	0/2	ND	0/3	0/3
SZ						
♂♂	5/5	4/6	6/6	4/5	6/6	9/10
♀♀	3/3	ND	4/4	3/5	3/3	5/5

ND, not done.

treated males, 200 islets free of round cell infiltrate ("0" insulitis) were scored. In all SZ-treated groups (cf. Fig. 4 *B–D*), far fewer islets >50 μm in diameter were observed, and a spectrum of round cell infiltration and islet degeneration was observed in all SZ-treated groups, irrespective of sex, blood glucose level, or presence or absence of a thymus. Pancreases showing the most severe islet loss were found in the group of 1-day Tx males treated with SZ; severe insulitis was found in four of six pancreases (Table 1), whereas in the other two pancreases, islet degeneration was almost total, with only a residual, weak insulitis observed around the few small clusters of aldehyde fuchsin-negative islet cells that remained.

As shown in Figs. 3 and 4, islet degeneration was associated with a very heavy insulitis. The presence of severe insulitis did not necessarily correlate with hyperglycemia, especially in SZ-treated females. For example, although mean blood glucose in the group of SZ-treated 2-day Tx females was only 123 + 36 mg/dl (citrate buffer control = 129 mg/dl), three of five (60%) of these mice had severe insulitis (Table 1). Indeed, of the group of unoperated SZ-treated mice shown in Fig. 1, 9 females and 10 males were autopsied at day 14. The group of nine females was only moderately hyperglycemic at sacrifice (224 ± 8 mg/dl), yet eight exhibited a very severe insulitis (cf. Fig. 3*B*) compared to nine in the male group showing a much more pronounced hyperglycemia (313 ± 13 mg/dl). Nevertheless, very

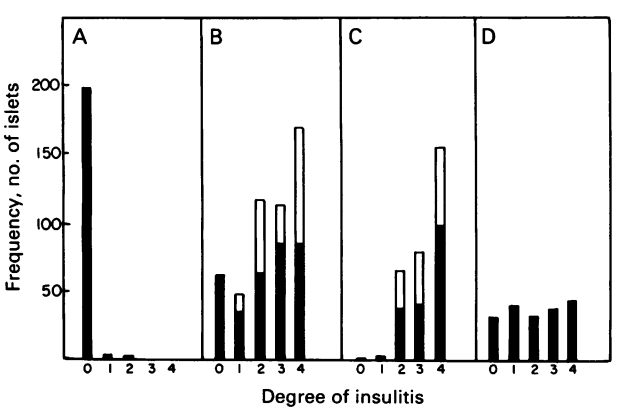

FIG. 4. Cumulative frequency of insulitis in BL/Ks male (solid bars) and female (open bars) mice as a function of treatment. Islets were scored for the presence of beta cell degeneration associated with a lymphocytic cell infiltrate according to the following criteria: 0 = no lymphoid cells, islet structure intact; 1 = <10 peri-insular lymphoid cells, islet structure intact; 2 = 10–20 peri-insular and intra-islet lymphoid cells, islet structure intact; 3 = >20 lymphoid cells widely distributed around and throughout islet (islet degenerate); 4 = severely degenerate islet with few, if any, beta cells surrounded by large numbers of lymphoid cells. (*A*) Day 1 ShTx, buffer control, n = 5 ♂♂; (*B*) No surgery, SZ, n = 10 ♂♂, 9 ♀♀; (*C*) Day 1 ShTx, SZ, n = 5 ♂♂, 3 ♀♀; (*D*) Day 1 Tx, SZ, n = 6 ♂♂.

heavy round cell infiltrates were found in islets scored in pancreases of four out of five females whose experimental day 14 blood glucose values were between 150 and 200 mg/dl (cf. Fig. 1).

Of interest was the finding of dysgenic ovaries in Tx females. Degenerate-appearing oocytes and follicle cells were observed in all SZ-treated females irrespective of thymus state but, in addition to small size and inactive appearance, follicular degeneration appeared more pronounced in Tx females. In the case of one 3-day Tx female, an acute oophoritis with heavy lymphocyte and granulocyte involvement was observed.

Just as normal or modestly elevated blood glucose values were not accurate predictors of the high frequency of SZ-induced insulitis in BL/Ks females, the induction of severe hyperglycemia in both BL/6 +/? and *nu/nu* mice conversely did not reflect an underlying insulitis. The finding by Rossini *et al.* (10) that the C57BL/6J inbred background was resistant to multiple low-dose SZ-induced insulitis was confirmed in this study; no evidence of round cell infiltrate was found in the pancreases of the three animals per genotype sampled at day 14 and day 35, respectively (cf. Fig. 1), although islets were very diminished in size and contained only small numbers of aldehyde fuchsin-positive beta cells.

DISCUSSION

The extent to which beta cell necrosis induced by the multiple, subdiabetogenic doses of SZ is due to the direct cytotoxic action of the drug or to cell-mediated immune cytolysis remains unknown. It seems clear that multiple low doses of SZ act cumulatively to exert a potent direct beta-cytotoxic action. The interaction between SZ and the beta cells appears to be a necessary step in pathogenesis. Thus, syngeneic islets implanted intrasplenically into healthy BL/Ks mice prior to induction of SZ insulitis suffered the same insulitis as observed in the host islets (13). However, when syngeneic islets were implanted intrasplenically 4 days after the last of five SZ injections, they were not destroyed as were the host islets and were able to cure the SZ-induced hyperglycemia (13).

Presumably SZ exerts its beta-cytotoxic action by lowering intracellular levels of NAD (14). Recent pharmacologic data suggest that this effect is mediated not via a block of NAD biosynthesis but rather by an SZ-induced increase in the activity of poly(ADP-ribose) synthetase, an important NAD-degrading enzyme (15). It has been well established that both genetic background and sex (e.g., maleness) are important modifiers of the diabetogenic action of SZ (6, 10, 16, 17); genetic background could conceivably control constitutive or inducible levels of the enzyme, while androgenic steroids could control enzyme induction at the transcriptional or translational level. Accordingly, inbred strains susceptible to SZ-induced diabetes might be expected to have higher levels of the enzyme than found in resistant strains. Alternatively, inbred strain- and sex-dependent sensitivity to SZ-induced diabetes could entail the induction of a different enzyme that degrades SZ itself to a more cytotoxic form (16).

It is considerably more difficult to account for inbred strain differences in susceptibility to SZ-induced insulitis. The present study shows that there is not necessarily a direct relationship between SZ-induced hyperglycemia and insulitis development. Both the Tx and nonTx BL/Ks female mice were relatively resistant to the SZ induction of hyperglycemia, but they did show widespread insulitis development. The converse was true in the euthymic +/? and athymic BL/6 *nude* male mice. In the case of the BL/Ks females showing strong insulitis but only weak hyperglycemia, the insulitis observed in eight of nine mice sampled at experimental day 14 did not presage onset of a more

severe hyperglycemia inasmuch as mean blood glucose of a group of 10 additional females aged to experimental day 35 remained at about the day 14 level (Fig. 1). Bonnevie-Nielsen *et al.* (18) have demonstrated that in SZ-treated BL/Ks males a major loss (>90%) in islet mass and beta cell function (85%) precedes not only the induction of hyperglycemia but also the onset of islet inflammation. That the relative resistance to SZ-diabetes shown by females may be mediated at the level of the beta cell's responsiveness to the direct cytotoxic action of SZ has been suggested by studies *in vitro* (19). Thus, while the destructive action of small doses of SZ appears to produce cumulative direct damage to beta cells prior to the appearance of insulitis, it cannot be deduced from the above studies that inflammatory reactions are not directly involved in beta cell destruction.

That the thymus plays an obligatory role in SZ-induced insulitis was based on circumstantial evidence from studies using genetically athymic BALB/c mice. An implied assumption underlying one of these studies was that the degree of SZ-induced hyperglycemia reflected the presence or absence of T cell-mediated immune reactions against beta cells (9). However, other reports confirm the conclusion reached in the present study, that hyperglycemia is not necessarily a consequence of SZ-induced insulitis, and vice versa. Kiesel *et al.* (20) transferred splenic lymphocytes from SZ-diabetic C57BL/6 BOM euthymic mice into congenic *nu/nu* mice; although this passive transfer failed to produce hyperglycemia or glucose intolerance in the *nude* mice, inflammatory cells were observed in or around the islets in 7 of 20 pancreases. Similarly, the presence of round cell infiltrates in or around islets, as well as variable degrees of abnormal glucose tolerance, but not fasting hyperglycemia, has been reported in young male and female mice known to be prone to autoimmune disease development [e.g., NZB, (NZB × NZB)F_1, and MRL-*lpr/lpr*] (21).

The experiments presented in the present study furnish no evidence to support an obligatory role for T-effector cell-mediated killing of beta cells in the multiple low-dose SZ model. However, because the Tx BL/Ks mice who developed insulitis were only examined visually for gross absence of the thymus and were not assessed by immunological methods for the complete absence of T cell-dependent immunoreactivity, it cannot be concluded with any certainty that T-effector cells were not involved in the inflammatory reactions associated with insulitis. The data suggest that, if T-effector cells played a role in the beta cell destruction, sufficient numbers of their thymus-derived precursors must have been dispersed to the lymphoid system early in the perinatal period. The experiments that used genetically athymic mice clearly demonstrated that the absence of an intact thymus from birth did not diminish the hyperglycemic action of SZ in mice on the C57BL/6 inbred background. However, because SZ-induced beta cell necrosis (by direct cytotoxicity) may be an event related to, but separable from, insulitis induction in susceptible strains such as BL/Ks, the hyperglycemia observed in the insulitis-resistant BL/6 *nu/nu* mice could be ascribed to direct SZ cytotoxicity.

Histological examination of the pancreatic islets of BL/Ks Tx mice treated with SZ revealed that islet destruction was at least as severe, if not *more* severe, than in the thymus-intact SZ-treated mice. Interestingly, various autoimmune disorders of ovary, thyroid, stomach, testis, and prostate can actually be induced by early thymectomy of inbred mice (22) and rats (23). This finding has led to the proposal that the seeding of helper and effector, but not suppressor, T cells into lymphoid organs can occur prior to day 3 thymectomy, such that immunological tolerance to organ-specific "self" antigens is no longer supported by the suppression of autoreactive effectors (22).

In the present study, ovarian dysgenesis characterized by severe atresia and follicle cell degeneration was observed in nearly all of the day 1 to day 3 Tx females, and one case of severe oophoritis was documented in a day 3 Tx female. Therefore, it may be of value to view normal thymus-dependent immunity as partially protective in this diabetes model. Consistent with this view is the report that impairment of normal immune regulation by induction of a graft-versus-host reaction resulted in round cell infiltration in pancreatic islets (24). This phenomenon could be explained by the release of autoreactive lymphocytes against islet cells as a consequence of reduced suppressor cell activity (24).

Modifying genes in the genetic background of the mouse and sex factors together—rather than the thymus gland—appear to be the more important determinants of intrinsic sensitivity or resistance to SZ-induced diabetes accompanied by insulitis. Consequently, caution should be exercised in extrapolating findings of decreased or negative responsiveness to SZ by *nude* mice to events at the immunological level, and particularly when the *nu* gene is studied on insulitis-resistant inbred C57BL/6 and BALB/c backgrounds.

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