β-Cell Function in Mice Injected with Mononuclear Splenocytes from Multiple-Dose Streptozotocin Diabetic Mice

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M. ARATA,* L. FABIANO DE BRUNO,* W. M. GONCALVEZ VOLPINI, J. C. QUINTANS,†
V. G. D'ALESSANDRO,* M. BRAUN,‡ AND J. C. BASABE*,[1]

Centro de Investigaciones Endocrinologicas (CEDIE), Hospital de Niños "Dr. R. Gutiérrez"; Departamento de Radiologia,† Comisión Nacional de Energia Atómica (CNEA); and Cátedra de Inmunología,‡ Facultad de Ciencias Veterinarias Universidad Nacional de Buenos Aires, Buenos Aires, Argentina*

Abstract. **Multiple low doses of streptozotocin (mld sz 40 mg/kg/day, five consecutive days) induce autoimmune diabetes in mice. The aim of the present work was to study β-cell function in mice injected with splenocytes from mld-sz diabetic mice. Mononuclear splenocytes (MS) from control or diabetic donors were injected into syngeneic C57BL/6J healthy mice (5×10^7 MS, ip). MS from diabetic donors did not produce basal hyperglycemia, but they induced abnormal ip glucose tolerance in recipient mice. These "diabetic" MS were also preferentially trapped by the recipient's pancreas. Perifused pancreas from mice injected with MS from mld sz-diabetic donors showed a diminished first and second phase of glucose-induced insulin secretion after 15 days of the cell injection. At this time, pancreatic insulin content among MS recipients did not differ from that found in controls or diabetic donors. Diabetic MS treated with Mitomycin C prior to transfer did not inhibit insulin secretion in recipient mice. Injection of MS from mice made diabetic by a single high sz dose (200 mg/kg) did not induce any alterations of the insulin secretion in recipients.**

There is enough evidence when using athymic and euthymic (BALB/c nu/nu and +/nu) mice to suggest that proliferation of the injected splenocytes enhanced the progression to the diabetic state, and that both donor and recipient T lymphocytes played an important part in this progression.

The results suggest that injection of MS from mld sz–diabetic mice interfere with glucose-stimulated insulin secretion in recipient mice and provide a basis for the study of the mechanisms involved in the onset and modulation of autoimmune pancreatic aggression. [P.S.E.B.M. 1994, Vol 206]

Type I (insulin-dependent) diabetes mellitus is an heterogeneous disorder of autoimmune etiology. Although the current mechanisms leading to pancreatic β-cell destruction remain unclear, it is well known that immune factors are strongly involved (1, 2). Diabetes can be induced experimentally in mice by mld sz which develops a diabetic syndrome characterized by a delayed, though progressive, hyperglycemia, insulitis, and severe destruction of β cells (3). In order to prove the autoimmune component of this syndrome, Buschard *et al.* (4) transferred diabetes by injecting MS from mld sz–induced diabetic mice to normal recipients. These animals developed hyperglycemia and insulitis. Kiesel *et al.* (5) and Paik *et al.* (6) also reproduced this experimental model. Several workers transferred diabetes by injecting splenocytes from genetically diabetic donors such as BB rats (7) and NOD mice (8). However, none of the transfer studies mentioned above gave any information regarding pancreatic function in recipient animals.

In the present study, we explore β-cell function in

[1] To whom requests for reprints should be addressed at Centro de Investigaciones Endocrinológicas (CEDIE), Hospital de Niños "Dr. R. Gutiérrez," Gallo 1330, 1425 Buenos Aires, Argentina.

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healthy mice injected with MS from syngeneic mld sz–induced diabetic mice, an experimental model which provides the tools to study the pathogenetic and modulating mechanisms of cellular immune aggression and insulin secretion.

Materials and Methods

Animals. Male C57BL/6J and congenitally athymic and euthymic BALB/c (nu/nu, +/nu) mice, 8–12 weeks old, were obtained from Departamento de Radiologia, Comision Nacional de Energia Atómica (CNEA) (Buenos Aires, Argentina). The animals were maintained according to the NIH Guidelines for the Care and Use of Laboratory Animals.

Diabetes Induction in MS Donors. (a) Induction of diabetes by mld sz (Upjohn Company, Kalamazoo, Michigan, USA). Sz was dissolved in 0.1 *M* citrate buffer, pH 4.5, and injected ip into nonfasting mice within 5 min of dissolution. Each mouse received 40 mg sz/kg body wt in a 0.1-ml volume during five consecutive days. Controls were injected ip with 0.1 ml of citrate buffer/day. Fifteen days after the last injection, plasma glucose was determined, the mice were killed and their spleens used for the transfer experiments. (b) Induction of diabetes by a single high dose of sz. In another series of experiments, sz was dissolved as indicated and injected ip into nonfasting mice. Each mouse received 200 mg sz/kg of body weight in 0.1 ml citrate buffer as a single injection. Controls were injected ip with 0.1 ml citrate buffer. Fifteen days after the injection, the plasma glucose levels were measured, the mice were killed and their spleens used for the transfer experiments.

Transfer Procedures. Transfer experiments were performed according to Buschard *et al.* (4). Spleens from control and diabetic donors were aseptically removed, pooled, and homogenized using soft mechanical disruption in cold sterile saline solution. MS were obtained using a Ficoll-Hypaque gradient (Ficollpaque, Pharmacia, Uppsala, Sweden) and then washed three times with sterile saline solution. Viability was assayed by the Tripan blue exclusion test (9) and 5×10^7 viable MS from diabetic or control pools were injected ip using a 0.2-ml sterile saline solution into normal syngeneic mice. In some of the experiments (Table V), MS recipients received a single low-dose ip injection of 40 mg sz per kg body wt, in 0.1 *M* citrate buffer (0.1 ml) the day before the MS transfer.

Treatment of MS with Mitomycin C. In some experiments, MS were incubated, prior to transfer, with Mitomycin C (Sigma, St. Louis, MO, 1 $\mu g/10^6$ cells/ml, 20 min at 37°C in a 95% humidified air and a 5% CO_2 atmosphere) in order to block DNA duplication and cell replication. After washing three times with sterile saline solution, 5×10^7 viable MS were injected ip into each mouse in 0.1 ml sterile saline solution.

Activation of MS with Concanavalin A. MS from control or diabetic donors were cultured with Concanavalin A (Con-A) to evaluate the effect of their mitogenic activation on β-cell function. MS were cultured for 72 hr at 37°C in a 95% humidified air and 5% CO_2 atmosphere, in the presence of 10 $\mu g/10^6$ MS/ml Con-A (Sigma) in RPMI 1640 medium, supplemented with 10% fetal calf serum (Gibco, OH), 100 μg/ml streptomycin and 100 units/ml Penicillin (Gibco, Grand Islam, NY). After culture, MS were washed three times with a fresh medium, the viability required was estimated at 95%–98%, and the observed blasts were in the 5%–10% range. The cells were then injected ip into healthy syngeneic recipients using a dose equivalent to that used in the transfer experiments (5×10^7 viable cells in 0.2 ml RPMI medium supplemented as above).

Glucose Measurement. Plasma glucose levels were determined in recipient mice previous to splenocytes injection and after 8 and 16 days. Blood samples were collected from the retroorbital venous plexus by using microcapillary heparinized tubes. Plasma glucose concentration was assayed by the glucose-oxidase method (glycemia enzymatic kit; Wiener Lab, Argentina) using a Beckman DB-G Spectrophotometer (Beckman Instruments, Fullerton, CA).

Intraperitoneal Glucose Tolerance Test. Animals were fasted for 12 hr and a glucose tolerance test was done between 8:00 AM and 12:00 AM. Blood samples were taken in a 20% saline solution at 0, 30, 60, and 120 min after an injection of 2 mg of glucose/g body wt.

MS Trapping in C57BL/6J Recipient Mice. In order to study MS homing in recipient mice (10), spleens from C57BL/6J control and diabetic donors were removed and MS were isolated as described above. The erythrocytes were hemolyzed with 0.83% NH_4Cl (Mallinkrodt, Chemical Works, St. Louis, MO), for 8 min at 37°C. MS were washed three times with a RPMI 1640 medium supplemented with 10% fetal calf serum (Gibco, OH), 100 ng/ml streptomycin and 100 units/ml Penicillin (Gibco, Grand Island, NY), and incubated with 25 μCi of $Na_2{}^{51}CrO_4/10^8$ cell/ml (CNEA, Buenos Aires, Argentina) during 30 min at 37°C in a humidified atmosphere containing 95% air and 5% CO_2. After incubation, MS were washed three times with fresh medium and the concentration was adjusted to 1×10^8 viable cells/ml. Each mouse received an iv dose of 1×10^7 cells, in 0.1 ml of the medium. Recipient mice were killed 24 hr after injection, their organs removed, and ^{51}Cr activity measured in blood cells (obtained from 0.1 ml whole blood), plasma (0.1 ml), one kidney, liver, spleen, one lung, pancreas, and lymph nodes (mesenteric, inguinal, and axillary).

Results were expressed (for each organ) as:

% $Na_2{}^{51}CrO_4$ uptake = (cpm organ/cpm total)100 where cpm total = Σ cpm in every organ tested in each mouse.

MS from mld sz–induced diabetic mice were labeled with $Na_2{}^{51}CrO_4$ and lysed by freezing-thawing in distilled water to measure the rate of "free" ^{51}Cr uptake. After centrifugation, 0.2 ml of the supernatant was injected into C57BL/6J mice. In all cases, the recipients were killed 24 hr after injection, their organs removed, and $Na_2{}^{51}CrO_4$ activity measured as described.

Perifusion of Pancreas Slices. The technique used was that described by Burr *et al.* (11). Thin slices from the whole pancreas of a single mouse were used in each perifusion. Krebs-Ringer bicarbonate buffer supplemented with lg/dl bovine albumin (Fraction V; Sigma) and 3.3 m*M* glucose was used as the perifusion buffer. The buffer was gassed with 95% O_2 and 5% CO_2 and the pH was kept constant at 7.38–7.40. Perifusion flux was 1.8–2.2 ml/min. Proteolytic effect on hormone secretion was avoided by adding 1000 KIU Trasylol/ml (Bayer, Buenos Aires, Argentina) to the buffer and by collecting the samples on 0.25 *M* ethylene-diamine tetraacetic acid (EDTA, Mallinckrodt, St. Louis, MO) in tubes at 4°C which were immediately frozen at −20°C, until insulin determination by RIA. The samples were collected after an initial 15-min recovery period. Samples obtained at 0 min and 1 min were used for baseline determinations. A stimulus of 16.5 m*M* glucose was added between 2 and 40 min; the first phase of insulin secretion was measured between 3–7 min; the second phase between 10–40 min.

Insulin Content in Pancreas and Islets from MS Recipient Mice. Fifteen days after MS injection, C57BL/6J recipient mice were killed by cervical dislocation, and the pancreas were quickly removed. Pancreatic islets were isolated by collagenase digestion (12). Insulin content was determined according to Eizirik *et al.* (13); triplicate groups of 10 islets from each control or experimental mouse were placed in sealed glass vials and ultrasonically disrupted in 0.5 ml of distilled water. A fraction of the aqueous homogenate was mixed with acid-ethanol (0.18 *M* HCl, in a 96% [v/v] ethanol), and insulin was extracted overnight at 4°C. The insulin content of the extracts was subsequently measured by RIA. The same procedure was used to extract insulin from pancreas (expressed as mU/mg tissue).

Insulin RIA. Insulin was determined using the method of Herbert *et al.* (14). Pork monoiodine ^{125}I-insulin with high specific activity was obtained from CNEA (Buenos Aires, Argentina). Rat standard insulin was obtained from the Novo Research Laboratories (Denmark). The anti-pork insulin antibody proved to be sufficiently "nonspecific" as to allow pork-labeled insulin to be displaced by rat or mouse insulin. The method allows determinations within 5.0–800 μU/tube range. The insulin assay sensitivity was 0.5 μU/ml; the intra-assay coefficient of variation (CV) was 8.2%, 6.6%, and 5.1% for 1–5, 5–10, and 10–50 μU insulin/ml determination ranges, respectively; the interassay CV was 6.6%, 4.9%, and 5.2% for the given ranges.

Statistical Analysis. Results were expressed as mean ± SEM. To evaluate insulin secretion in perifused pancreatic slices, areas under the curves for insulin secretion were integrated. Statistical analysis of the data was performed by the two-tailed Student's test for unpaired samples and by the one-way ANOVA (15). Scheffe's test for multiple comparisons between individual groups was employed. A $P < 0.05$ was considered statistically significant.

Results

Effect of MS Injection on Recipient Glycemia Levels. Diabetic MS donors had a significant hyperglycemia (472 ± 15 mg/dl vs 135 ± 10 mg/dl for the control group; $P < 0.001$, $n = 20$ in each group). Glycemia was measured in syngeneic recipient mice 8 and 16 days after MS transfer from control or mld sz–diabetic donors. MS injection did not change glycemia levels in recipients, at either 8 or 16 days (after 8 days: 130 ± 9 mg/dl for control MS vs 136 ± 4 mg/dl for diabetic MS; after 16 days: 132 ± 4 mg/dl for control MS vs 132 ± 9 mg/dl for diabetic MS; $n = 6$ in all groups).

Intraperitoneal Glucose Tolerance Test in MS Recipient. MS from mld sz–diabetic donors induced significant hyperglycemia 120 min after glucose injection, both at 8 and 16 days after MS injection (after 8 days: 145 ± 15 mg/dl for control vs 254 ± 9 mg/dl for diabetic; after 16 days: 146.4 ± 9 for control vs 274 ± 4 for diabetic; $n = 6$ in all groups, $P < 0.001$ in both cases). In order to evaluate if this effect was due to transfer of immune cells unspecifically activated by a low dose of sz, another group of mice ($n = 8$) received MS from single high-dose sz–diabetic mice. No alteration of the glucose tolerance in recipient mice was observed.

Insulin Secretion in MS Recipient Mice. C57BL/6J recipient mice were sacrificed 15 days after cell injection and their pancreatic glands used for perifusion experiments. No significant differences were found in basal insulin secretion in both control and experimental groups (Table I). MS from mld sz–diabetic donors (Table I, Group 1) induced a significant impairment of first ($P < 0.05$) and second ($P < 0.02$) phases of glucose-stimulated insulin secretion. MS from single high-dose sz (shd sz) diabetic donors (Table I, Group 2) did not induce any alterations in the insulin secreted by recipient mice pancreas. Insulin content in pancreas and islets from mice injected with

Table I. Insulin Secretion from Perifused Pancreas Slices Stimulated by Glucose 16.5 mm

Group	Basal (μU/2 min/100 mg wt)	First phase (μU/4 min/100 mg wt)	Second phase (μU/30 min/100 mg wt)
1 Control (mld citrate MS recipients)	127 ± 10	974 ± 42	10077 ± 209
mld sz–Diabetic MS recipients	108 ± 9	630 ± 37[a]	8958 ± 251[b]
2 Control MS recipients	120 ± 8	903 ± 93	10050 ± 196
shd sz–Diabetic MS recipients	116 ± 11	926 ± 75	10142 ± 231
3 Control injected with mld citrate	112 ± 7	1005 ± 67	9979 ± 228
Injected with mld sz	118 ± 6	233 ± 26[a]	1523 ± 103[a]
4 Euthymic (+/nu) injected with mld citrate	115 ± 6	926 ± 72	10027 ± 202
Euthymic (+/nu) injected with mld sz	123 ± 8	256 ± 48[a]	1678 ± 112[a]
Athymic (nu/nu) injected with mld citrate	118 ± 8	974 ± 70	10092 ± 194
Athymic (nu/nu) injected with mld sz	110 ± 7	709 ± 50[b]	8577 ± 149[b]

Group 1: C57BL/6J recipient mice transferred with control or mld sz–diabetic MS; Group 2: C57BL/6J recipient mice transferred with control or shd sz–diabetic MS; Group 3: C57BL/6J mice 15 days after the last low-dose citrate or sz injection; Group 4: euthymic BALB/c (+/nu) and athymic BALB/c (nu/nu) mice 15 days after the last dose citrate or sz injection. Insulin secretion is expressed as areas under the curves; data are expressed as mean ± SEM; $n = 6$ in all groups; [a]$P < 0.05$, [b]$P < 0.02$, both compared with the control group.

MS from mld sz–diabetic donors showed no significant differences when compared with control group (2.17 ± 0.30 vs 2.07 ± 0.84 mU/mg and 2301 ± 132 vs 2368 ± 164 μU/islet respectively).

Insulin from mld sz–diabetic mice was included in the same table (Group 3) so the degree the disease was transferred can be judged.

MS Trapping in Recipient Mice. MS from mld sz–diabetic donors were labeled with $Na_2{}^{51}CrO_4$ and injected as indicated. Table II shows MS trapping in recipients 24 hr after cell injection. $Na_2{}^{51}CrO_4$ uptake was significantly increased in pancreas from mice injected with MS from mld sz–diabetic donors ($P < 0.005$) when compared with the control group. In the remaining organs, including plasma and blood cells, no significant differences among control and diabetic MS were found. $Na_2{}^{51}CrO_4$ distribution in mice injected with lysed MS is shown in Table II. The difference in the pattern of ^{51}Cr distribution observed in these animals indicates that $Na_2{}^{51}CrO_4$ activity in the previous groups was carried out by nondamaged viable MS.

T Lymphocytes' Role in MS Donor and Recipient Mice. Congenitally athymic BALB/c (nu/nu) and euthymic BALB/c (+/nu) mice were injected with mld sz as described above. Euthymic and athymic mice developed hyperglycemia 16 days post–mld sz injection. Nevertheless, euthymic mice glycemia levels were significantly higher when compared with athymic mice (474 ± 66 vs 183 ± 16 mg/dl respectively, $n = 6$ for each group $P < 0.01$). Table I, Group 4 shows insulin secretion in these groups; euthymic mld sz–injected mice showed significantly diminished first and second phases of glucose-induced insulin secretion. Athymic mice also showed diminished first and second phases of insulin secretion, but these decrements were significantly lower than those observed in euthymic mice ($P < 0.05$). These results indicate that T lymphocytes from mld sz–injected mice should be involved in

Table II. Splenocytes Homing in C57BL/6J Mice

Organ	% $Na_2{}^{51}CrO_4$ uptake		
	Control splenocytes	Diabetic splenocytes	Lysated[b] splenocytes supernatant
Plasma (0.1 ml)	0.47 ± 0.14	0.53 ± 0.21	5.08 ± 0.14
Blood cells	3.64 ± 0.33	3.21 ± 0.31	0.17 ± 0.04
Pancreas	0.62 ± 0.04	1.32 ± 0.16[a]	0.09 ± 0.03
Lung	1.72 ± 0.18	1.97 ± 0.14	0.81 ± 0.04
Spleen	55.71 ± 3.43	55.29 ± 2.09	8.69 ± 1.32
Liver	18.59 ± 1.79	19.32 ± 0.39	50.55 ± 1.46
Kidney	14.34 ± 1.51	13.23 ± 1.67	33.68 ± 0.73
Lymph nodes	4.91 ± 0.28	5.13 ± 0.63	0.93 ± 0.12

Results are expressed as % $Na_2{}^{51}CrO_4$ uptake = (cpm $Na_2{}^{51}CrO_4$ organ/cpm $Na_2{}^{51}CrO_4$ total measured × 100). Number of recipient mice = 22 in each group. [a]$P < 0.05$ when compared with control splenocytes; [b]cpm statistically different in every organ when compared with the diabetic or control splenocytes groups.

some amplificatory mechanism of sz-induced pancreatic injury. Furthermore, these results allow a better judgment of how important the thymus is for mld sz–induced β-cell damage.

In order to evaluate if donor T lymphocytes were implicated in the insulin secretion impairment observed in the recipient mice, transfer experiments were done using euthymic (BALB/c +/nu) recipient mice (Table III). Transfer of MS from mld sz–injected euthymic mice produced a greater impairment of insulin secretion than MS from mld sz–injected athymic mice ($P < 0.05$).

In another group of mice, transfer experiments were done as above, but using euthymic and athymic recipient mice (Table IV). Only MS from BALB/c +/nu induce insulin secretion impairment in euthymic and athymic recipient mice. However, when MS recipients were athymic mice, a lower extent of insulin secretion impairment was noted.

Taken together these results provide evidence for a T-cell component in mld sz–induction of diabetes and a donor and recipient T-cell component in the adoptive transfer of "diabetes."

Modulations of Insulin Secretion in Recipient Mice Transferred with MS from mld sz–Diabetic Mice. To block DNA duplication and cell replication, MS from C57BL/6J control or mld sz–diabetic mice were incubated with Mitomycin C prior to transfer. After incubation, viability was estimated at 95%. Mitomycin C treatment completely abolished the effect of MS from mld sz–diabetic donors on glucose-induced insulin secretion in syngeneic healthy recipient mice (Table V, Group 2).

When MS from mld sz–diabetic mice were cultured with Con A, they induced a greater impairment in insulin secreted by recipient mice (Table V, Group 3) with the development of hyperglycemia (293 ± 23 mg/dl vs 142 ± 10 in the absence of Con A, $P < 0.01$).

To facilitate β-cell recognition by MS from mld sz–diabetic donors, recipient mice were injected with a single dose of sz (40 mg/kg body wt) 24 hr before MS transfer. This sz dose did not cause, per se, either hyperglycemia or insulin secretion impairment in recipient mice (data not shown). Transfer of MS from mld sz–diabetic donors induced an insulin secretion impairment significantly higher than in naive syngeneic mice (Table V, Group 4). In this case, recipients developed basal hyperglycemia 8 days post MS injection (307 ± 17 mg/dl vs 137 ± 9 when not injected with a single dose of sz, $P < 0.001$). All the other groups in Table V, were normoglycemic (128–149 mg/dl).

Discussion

The data presented here show that injection of MS from mld sz–diabetic mice induced abnormal glucose tolerance and insulin secretion impairment in healthy syngeneic recipients. Buschard *et al.* (4) reported diabetes transfer by injection of splenocytes from mld sz–induced diabetic mice. The authors described that hyperglycemia and insulitis developed into naive syngeneic recipient mice. In their experimental model, pancreatic immune aggression was T-lymphocyte dependent (16).

Several authors reproduced this experimental model with different results. Kiesel *et al.* (17) reported that insulitis and abnormal glucose tolerance in recipients were a strain-dependent phenomena. Paik *et al.* (6) induced only transient glucose intolerance in diabetic splenocyte recipients and confirmed that the effect was mediated by T lymphocytes. However, none of the above mentioned groups measured insulin secreted from recipient mice pancreas. In the present study insulin secretion alterations showed to be the most sensitive evidence of β-cell function impairment. In different experimental models of diabetes, as well as in human pathology, a progressive loss in β-cell mass and an evolutive decrease in insulin secretion were observed before glucose intolerance and hyperglycemia developed (18, 19, 20). In our experimental condition, although insulin secretion decreased and glucose intolerance was seen in recipients of MS from mld sz–diabetic mice, the extent of pancreatic alteration did not seem to be enough to cause basal hyperglycemia. Furthermore, β-cell impairment seems to be a functional alteration, as suggested by the fact that there was no difference in pancreatic insulin content among recipients of control or "diabetic" MS. This is further supported by the fact that acid-ethanol extrac-

Table III. Insulin Secreted by Perifused Pancreatic Slices from Euthymic Mice (BALB/c +/nu) Transferred with MS from Euthymic or Athymic Mice, Previously Injected with mld Citrate or sz

MS donor	Previous treatment	Insulin secretion		
		Basal (μU/2 min/100 mg wt)	First phase (μU/4 min/100 mg wt)	Second phase (μU/30 min/100 mg wt)
Euthymic mice	mld citrate	119 ± 6	936 ± 38	9299 ± 305
	mld sz	115 ± 10	626 ± 40[a]	6539 ± 176[a]
Athymic	mld citrate	120 ± 8	922 ± 21	9222 ± 150
	mld sz	113 ± 10	796 ± 26[a,b]	7987 ± 200[a,b]

[a] $P < 0.05$ compared with control; [b] $P < 0.05$ compared with mld sz euthymic mice group; $n = 6$ in all cases.

Table IV. Insulin Secreted by Perifused Pancreatic Slices from Euthymic BALB/c (+/nu) or Athymic BALB/c (nu/nu) Recipient Mice Transferred with Control or Diabetic MS from Euthymic or Athymic Mice

MS recipient	MS donor	Insulin secretion		
		Basal (μU/2 min/ 100 mg wt)	First phase (μU/4 min/ 100 mg wt)	Second phase (μU/30 min/ 100 mg wt)
BALB/c +/nu	Control BALB/c +/nu	108 ± 21	908 ± 27	9100 ± 177
	mld sz diabetic BALB/c +/nu	110 ± 15	631 ± 60[a]	7298 ± 136[a]
BALB/c nu/nu	Control BALB/c +/nu	109 ± 22	916 ± 39	9246 ± 140
	mld sz diabetic BALB/c +/nu	121 ± 21	788 ± 52[a]	9108 ± 100
	mld sz diabetic BALB/c nu/nu	117 ± 8	929 ± 31	9011 ± 123

[a] $P < 0.05$ compared with control; $n = 6$ in all cases.

Table V. Insulin Secreted by Perifused Pancreatic Slices from Recipient Mice Transferred with MS from Control or mld sz–Treated Mice Previously Modulated by Mytomicin C Treatment, Con-A, or a Single Low sz Injection (sz)

Group	Insulin secretion		
	Basal (μU/2 min/100 mg wt)	First phase (μU/4 min/100 mg wt)	Second phase (μU/30 min/100 mg wt)
1 Control MS	107 ± 12	974 ± 42	10200 ± 221
	$n = 18$	$n = 18$	$n = 18$
mld sz MS	110 ± 13	639 ± 33[a]	8971 ± 231[b]
	$n = 18$	$n = 18$	$n = 18$
2 Mitomycin C–incubated control MS	120 ± 11	965 ± 70	10126 ± 191
	$n = 6$	$n = 6$	$n = 6$
Mitomycin C–incubated mld sz MS	107 ± 9	956 ± 38	10240 ± 106
	$n = 6$	$n = 6$	$n = 6$
3 Con-A cultured control MS	100 ± 18	1015 ± 61	10293 ± 460
	$n = 6$	$n = 6$	$n = 6$
Con-A cultured mld sz MS	130 ± 20	542 ± 21[c,e]	7766 ± 172[b,d]
	$n = 6$	$n = 6$	$n = 6$
4 sz + control MS	127 ± 10	986 ± 69	10467 ± 139
	$n = 6$	$n = 6$	$n = 6$
sz + mld sz MS	119 ± 15	504 ± 16[c,e]	8426 ± 79[b,e]
	$n = 6$	$n = 6$	$n = 6$

[a] $P < 0.05$ compared with control; [b] $P < 0.01$ compared with control; [c] $P < 0.001$ compared with control; [d] $P < 0.05$ compared with mld sz MS; [e] $P < 0.01$ compared with mld sz MS.

tion of insulin from pancreas has been shown to correlate with β-cell mass (20). Mitomycin C treatment of MS from diabetic donors, prior to transfer, completely abolished their effect on recipients, suggesting that this effect implicates transferred splenocyte proliferation in recipient mice. Furthermore, MS from mld sz–diabetic donors showed a specific homing towards pancreas which might indicate an early stage in the immune aggression phenomenon. These results (mitomycin C and homing) suggest that, in this experimental model, transferred MS play a main role in the impairment of β-cell function. Donor T lymphocytes seemed to be critically involved, since MS from mld sz–injected athymic donors could not induce any alterations in recipient mice. Moreover, a donor T MS/recipient T lymphocyte interaction may be proposed, since the extent of insulin secretion impairment was lower when MS recipients were athymic mice.

Gleichman *et al.* (21) reported that sz could have an antigenic effect per se. In the present transfer studies, a direct toxic effect of sz on recipient mice pancreas or on donor splenocytes, leading to sz-specific immune reactions that facilitate β-cell injury could be ruled out. Results clearly show that single high dose of sz in MS donors did not induce any alterations in insulin secretion from MS recipients.

Insulin secretion impairment could also be modulated. A single low dose of sz injected in MS recipients prior to control cell transfer did not induce any insulin impairment or hyperglycemia. However, subsequent diabetic MS transfer elicited a greater insulin impairment in recipient animals. We are tempted to speculate that donor MS primed *in vivo* towards sz-altered β-cell, recognize in recipients a sz-modified β-cell, and then strongly react. Also, it has been postulated that sz could induce Class II antigen expression in β cells (22).

As a consequence, a single low dose of sz in MS recipients might facilitate β recognition by MS. Previous culture of diabetic MS with Con-A induced a greater extent of insulin secretion impairment in MS recipients which then developed hyperglycemia. As Con-A is a polyclonal mitogenic agent (23), an unspecific equal expansion of lymphocytes could also induce an increase in anti-β-cell effectors and therefore functional β-cell alterations become more intensive; beside this, an activation of anti-β-cell aggression should not be ruled out. These results also support that additional β-cell aggression by some environmental insults might precipitate the onset of hyperglycemia or overt diabetes as described by Ihm *et al.* in the NOD mice (24).

Syngeneic transfer of autoimmune diabetes has been reported in other experimental models as the NOD mouse and BB rat (7, 8, 25). However, there are some difference between NOD mice, BB rat and the experimental model described here. In those models, autoimmune diabetes develops spontaneously and insulitis preceeds by several weeks the onset of clinically overt diabetes (25). In both cases adoptive transfer of diabetes was achieved in diabetes prone recipients, which presumably have already begun to self-damage their pancreatic islets. This fact limits the use of these models when studying the mechanisms of pancreatic immune destruction, especially when relating to its early stages. Furthermore, diabetes transfer was obtained in immunosuppressed recipients or by previous expansion of donor MS (7, 8).

This experimental model in which insulin secretion impairment was induced in healthy naive recipient mice by MS from mld sz–diabetic donors, appears to be a useful system to study mechanisms involved in the onset and modulation of autoimmune pancreatic aggression.

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