Inhibition of Nitric Oxide Generation: Normalization of In Vitro Insulin Secretion in Mice With Multiple Low-Dose Streptozotocin and in Mice Injected With Mononuclear Splenocytes From Diabetic Syngeneic Donors

L.M. Karabatas, L. Fabiano de Bruno, C.F. Pastorale, C. Cullen, and J.C. Basabe

We studied the effect on in vitro glucose-induced insulin secretion of in vivo administration of L-N^G-monomethyl-arginine (L-NMMA), a competitive inhibitor of nitric oxide (NO) synthase, to mice injected with multiple low-dose streptozotocin (mld-SZ). In addition, the effect of L-NMMA treatment on the capacity of mononuclear spleen cells (MS) from mld-SZ mice to transfer alterations in insulin secretion from normal syngeneic receptors was also investigated. We also studied the effect of in vivo treatment with L-NMMA on anti-β-cell cellular immune aggression (CIA) by coculturing MS from mld-SZ mice with rat dispersed islet cells. Our results show that mld-SZ mice treated with 0.25 mg L-NMMA/g body weight had normoglycemia, first- and second-phase glucose-stimulated insulin secretion similar to those obtained in nondiabetic mice—effects not observed with a lower dose of L-NMMA (0.17 mg/g body weight)—and a diminished anti-β-cell CIA. We also demonstrate that mice injected with MS from syngeneic donors treated with mld-SZ plus 0.25 mg L-NMMA/g had normal levels for first-phase glucose-stimulated insulin secretion and an absence of CIA. Taken together, these findings seem to indicate that prevention of in vivo NO production may block the onset of diabetes in mld-SZ mice, and that L-NMMA administration to diabetic donor mice prevents inhibition of first-phase insulin secretion and CIA in the transferred recipient mice. Although a nonimmunological mechanism or mechanisms of diabetes prevention by L-NMMA cannot be excluded, these results suggest that L-NMMA treatment could also be acting on T-cell–dependent immune reactions.

THE DIABETIC SYNDROME induced in mice by multiple low-dose streptozotocin (mld-SZ) is due not only to a β-cytotoxic effect of the drug, but mainly to a subsequent immune destruction with an inflammatory process leading to macrophage and lymphocyte infiltration with concomitant β-cell lysis.[1,2]

In animal models of immune-mediated diabetes, macrophages have been demonstrated to be among the first inflammatory cells to infiltrate the islet,[3] secreting cytokines such as interleukin-1 (IL-1) and tumor necrosis factor, which lead to an impairment of β-cell function followed by cell death.[2] Macrophages are also the effector cells in islet cell lysis in vitro,[4] and kill syngeneic pancreatic islet cells in vitro via arginine-dependent nitric oxide (NO) generation.[5]

NO synthase, which has two different isoforms (either constitutive or cytokine-inducible),[6,7] catalyzes the mixed functional oxidation of L-arginine to yield L-citrulline and NO. Several studies have demonstrated that IL-1 induces the expression of NO synthase, increasing NO formation by the islets of Langerhans with the subsequent inhibition of mitochondrial function and DNA synthesis and thereby resulting in β-cell death.[4,6,7]

Analogs of L-arginine such as L-N^G-monomethyl-arginine (L-NMMA) competitively inhibit both isoforms of NO synthase.[8] It has also been reported that mld-SZ–injected mice treated with L-NMMA showed little or no cellular infiltration in the islets and a lower degree of islet cell destruction.[9] In the present report, we studied the effect of in vivo treatment with L-NMMA on in vitro insulin secretion patterns from mld-SZ–injected mice and the capacity of mononuclear spleen cells (MS) from mld-SZ mice to exert anti-β-cell cellular immune aggression (CIA). In addition, MS isolated from these mld-SZ + L-NMMA–treated mice were transferred to normal syngeneic receptors to investigate the ability of these cells to impair in vitro insulin secretion in the recipient normal mice. MS from recipient mice were also tested for CIA.

MATERIALS AND METHODS

Male Wistar rats weighing 150 to 200 g and male C3H inbred mice weighing 20 to 24 g were obtained from the Comisión Nacional de Energía Atómica, Buenos Aires, Argentina, and housed in a room with a controlled temperature (23°C) and fixed artificial light-dark cycle. They had free access to water and a standard laboratory chow.

Mice were randomly divided into five experimental group: group 1, mice injected intraperitoneally (IP) with 0.1 mL citrate buffer, pH 4.5, for 5 consecutive days (days 1 to 5) and with 0.2 mL phosphate-buffered saline (PBS) for another 5 consecutive days (days 6 to 10); group 2, mice injected IP with 40 mg/kg body weight SZ (Sigma, St Louis, MO) dissolved in citrate buffer, pH 4.5, for 5 consecutive days, and thereafter with five consecutive IP injections of PBS from day 6 to day 10; group 3, mice treated with five injections of SZ (days 1 to 5) and thereafter with five injections of 0.17 mg L-NMMA/g body weight in 0.2 mL PBS (days 6 to 10); group 4, mice treated similarly to the third group, but with five injections of 0.25 mg L-NMMA/g body weight; and group 5, mice treated with five injections of SZ (days 1 to 5) and thereafter with five injections of L-arginine (0.25 mg/g body weight in 0.2 mL PBS) (days 6 to 10).

Blood samples for determination of plasma glucose levels were obtained by retro-orbital sinus puncture in nonfasted mice on days 0 and 28.

From the Laboratorio de Diabetes Experimental, Centro de Investigaciones Endocrinológicas, Hospital de Niños "Dr. R. Gutierrez," Buenos Aires, Argentina.

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Address reprint requests to J.C. Basabe, MD, Centro de Investigaciones Endocrinológicas, Hospital de Niños "Dr. R. Gutierrez", Gallo 1330, 1425, Buenos Aires, Argentina.

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Animals were killed by cervical dislocation on day 29, and the pancreas and spleen were removed for perifusion of pancreatic slices and isolation of MS, respectively.

mld-SZ Mice Treated With L-NMMA

Insulin secretion from perifused pancreatic slices. Pancreata obtained from mice belonging to the five experimental groups were used in the perifusion studies. The technique described by Burr et al[10] with slight modifications[11] was used. Krebs-Ringer bicarbonate buffer supplemented with 10 mg/mL dextran 70 (Sigma), 3.3 mmol/L glucose, and 100 KIU Trasylol/mL (Bayer, Buenos Aires, Argentina) was used as the perifusion buffer. The pH of the buffer, kept under constant 95% O_2/5% CO_2 gassing, was 7.38 to 7.40. Thin slices from a whole pancreas of a single mouse were used in each perifusion. The samples were collected following an initial 15-minute recuperation period in tubes kept at 4°C and containing 0.2 mL 0.25-mol/L EDTA. The samples were immediately frozen at −20°C. Samples from minutes 1 and 2 were used for baseline determinations. A stimulus of 16.5 mmol/L glucose was added to the perifusion buffer from minute 3 to 40. Perifusion flux was 1.8 to 2.2 mL/min.

CIA. Spleens from mice treated with citrate + PBS, mld-SZ + PBS, and mld-SZ + L-NMMA 0.25 mg/g were shredded with a wire mesh followed by rinsing twice in sterile saline solution[12,13] and suspended in minimal essential medium (Gibco, Paisley, UK) modified (as described later) to reach a final concentration of 4×10^6 MS/mL. Viability of MS was assayed by the trypan blue exclusion test,[14] and only suspensions having at least 90% viable MS were used.

Islets of Langerhans were obtained from collagenase-treated (Serva Feinbiochem, Heidelberg, Germany) adult Wistar rat pancreas according to the method of Lacy and Kostianovsky.[15] To obtain islet cell suspensions, freshly isolated islets were treated with EDTA and trypsin as described by Ono et al.[16] Islet cells were suspended in basal minimal essential medium (5.5 mmol/L glucose) with Eagle salts supplemented with 10% fetal calf serum, 2 mmol/L glutamine, 1 mmol/L sodium pyruvate, 0.814 mg/L nonessential amino acids (Gibco, Paisley, UK), and 100 U/mL penicillin. Cell viability was estimated by the trypan blue exclusion test, and only suspensions having at least 90% viable cells were used.

Islet cell stimulation[17,18] was performed as follows. The islet cell suspension was placed in 96-well Falcon microtest plates (Becton Dickinson, Oxnard, CA) at 5×10^3 cells per well, with addition of MS from a single mouse of one of the different experimental groups (4×10^5 MS per well in 100 μL) or basal medium (100 μL), and incubated 18 hours. Ten wells were used for each mouse. At the end of this incubation period, the wells were carefully washed and the supernatant was collected to assess prestimulatory insulin release. The medium was replaced with 200 μL basal medium (glucose 5.5 mmol/L) or 200 μL stimulatory medium (glucose 16.5 mmol/L plus theophylline 5.5 mmol/L). Five wells were used for each basal or stimulatory medium. MS remained with the cells during both basal and stimulatory periods. Supernatants were withdrawn after 5 minutes and rapidly frozen for insulin determinations.

Net basal secretion is presented as the insulin secretion (microunits per 5,000 cells) during a 5-minute incubation period in the presence of basal medium minus the prestimulatory secretion, and net stimulated secretion is presented for the same 5-minute period in stimulatory medium minus the prestimulatory secretion. The real stimulated insulin secretion is presented for the net stimulated secretion minus the net basal secretion.

The reproducibility of CIA test results (real stimulated secretion) had 4.01% interassay and 4.23% intraassay coefficients of variation.

Passive Transfer of MS From mld-SZ Mice Treated With L-NMMA

Mice from the five experimental groups were used as MS donors. MS were aseptically isolated by shredding the spleens with a wire mesh followed by rinsing twice in sterile saline solution.[12,13] Viability of MS was assayed by the trypan blue exclusion test,[14] and only suspensions having at least 90% viable MS were used.

Five groups of syngeneic normal mice were injected IP with 5×10^7 viable MS (in 0.2 mL sterile saline solution) obtained from the five groups of donor mice. Recipient mice were killed 15 days after the transfer procedure, and their pancreata were removed. Islets of Langerhans were isolated by collagenase digestion[15] and perifused (40 islets per chamber) to study insulin secretion patterns. The perifusion technique was the same as described earlier, but the perifusion flux was 0.8 to 1.0 mL/min.

CIA was performed as previously described, but with MS from transferred animals.

Analytical Methods

Nonfasted serum glucose levels were determined using a Glicemia Enzimatica Kit (Wiener Laboratory, Buenos Aires, Argentina). Insulin was determined in the samples from perifusion and from CIA by the method of Herbert et al.[19] Pork monoiodine ^{125}I-insulin was obtained from CNEA (Buenos Aires, Argentina). Rat standard insulin was obtained from Novo Research Laboratories (Bagsvaerd, Denmark). Guinea pig antiporcine insulin antiserum was sufficiently nonspecific as to allow pork labeled insulin to be displaced by mouse insulin.

The insulin assay sensitivity was 2.5 μU/mL. The intraassay coefficient of variation was 8.7%, 6.2%, and 5.1% for 1 to 5, 5 to 10, and 10 to 50 μU insulin/mL, respectively. The interassay coefficient of variation was 6.6%, 5.0%, and 5.2% for the given ranges.

Statistical Analysis

To evaluate insulin secretion from perifused pancreatic slices or islets, we integrated the areas under the stimulated insulin secretion curves. For pancreatic slices, the first secretory peak was integrated between minutes 3 and 8, and for islets, between minutes 3 and 7, and the second peak between minutes 9 and 40 of perifusion for both cases. Statistical analysis of the data was performed with ANOVA and Scheffe's test. All results are expressed as the mean ± SEM.

RESULTS

Treatment With L-NMMA in mld-SZ Mice: Serum Glucose, Insulin Secretion, and CIA

Table 1 shows the values for nonfasted serum glucose levels in the five experimental groups of mice described previously. Mice injected with mld-SZ and then treated with PBS, 0.17 mg L-NMMA/g, or 0.25 mg L-arginine/g body weight (groups 2, 3, and 5) had significant increments in serum glucose values compared with nondiabetic animals (group 1). However, treatment with 0.25 mg L-NMMA/g completely prevented the development of hyperglycemia in SZ-injected mice (group 4).

Basal insulin secretion from perifused pancreatic slices was not significantly different in the five experimental

Table 1. Nonfasted Serum Glucose Levels (mg/dL) in mld-SZ or Control Mice

Group	Day 0	Day 28
Citrate + PBS	158.5 ± 6.2, n = 6	159.7 ± 5.4, n = 6
mld-SZ + PBS	167.6 ± 7.5, n = 7	577.3 ± 30.1, n = 7*†‡
mld-SZ + L-NMMA 0.17 mg/g	165.3 ± 5.1, n = 6	559.1 ± 61.9, n = 6*†‡
mld-SZ + L-NMMA 0.25 mg/g	164.1 ± 4.0, n = 8	178.1 ± 8.1, n = 8
mld-SZ + L-Arg 0.25 mg/g	160.4 ± 4.9, n = 7	428.4 ± 41.4, n = 7*†‡

*P < .01 *v* day 0.
†P < .01 *v* group 1 at day 29.
‡P < .01 *v* group 4 at day 29.

groups (group 1, 49.83 ± 2.21 μU/min/100 mg wet tissue, n = 6; group 2, 40.14 ± 1.92, n = 7; group 3, 40.33 ± 3.55, n = 6; group 4, 46.94 ± 2.66, n = 8; and group 5, 37.86 ± 2.05, n = 7).

Areas under the first-phase glucose-stimulated insulin secretion from perifused pancreatic slices showed significant diminution in all SZ-treated groups, except for mld-SZ mice injected with the higher dose of L-NMMA (0.25 mg/g), whose levels did not differ significantly from those in nondiabetic mice (Fig 1).

Second-phase glucose-stimulated insulin secretion was also significantly diminished in mld-SZ diabetic mice from groups 2, 3, and 5 (Fig 2). In contrast, when diabetic mice were injected with 0.25 mg/g L-NMMA (group 4), the levels attained did not differ from those observed in nondiabetic mice.

Figure 3 was included to show the waveforms of stimulated insulin secretion in perifused pancreatic slices from nondiabetic mice (group 1), mld-SZ mice (group 2), and mld-SZ mice with five injections of 0.25 mg/g L-NMMA (group 4).

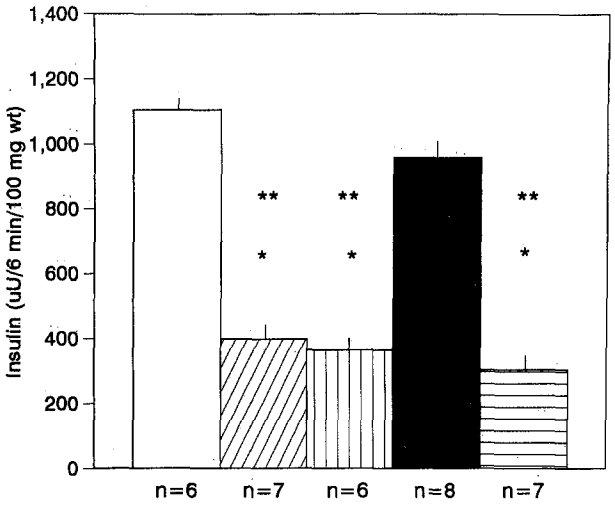

Fig 1. Areas under the first-phase glucose-induced insulin secretion in perifused pancreatic slices from mice injected with citrate buffer + PBS (□), mld-SZ + PBS (▨), mld-SZ + 0.17 mg/g L-NMMA (▥), mld-SZ + 0.25 mg/g L-NMMA (■), and mld-SZ + 0.25 mg/g L-arginine (▤). Results are the ±SEM. *P < .01 *v* mice injected with citrate buffer + PBS; **P < .01 *v* mice injected with mld-SZ + 0.25 mg/g L-NMMA.

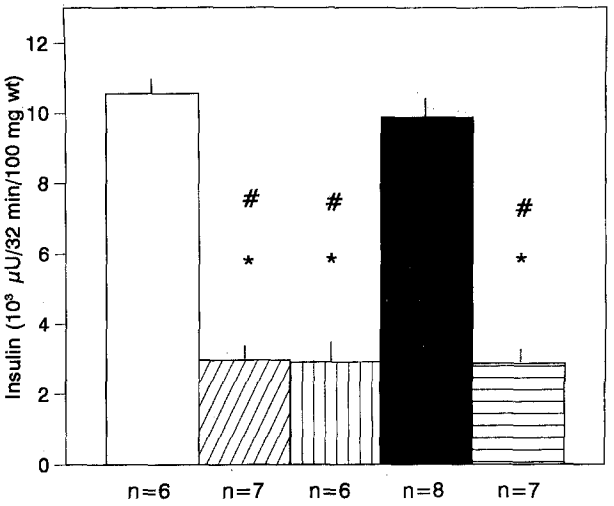

Fig 2. Areas under the second-phase glucose-stimulated insulin secretion in perifused pancreatic slices from mice injected with citrate buffer + PBS (□), mld-SZ + PBS (▨), mld-SZ + 0.17 mg/g L-NMMA (▥), mld-SZ + 0.25 mg/g L-NMMA (■), and mld-SZ + 0.25 mg/g L-arginine (▤). *P < .01 *v* mice injected with citrate buffer + PBS; #P < .01 *v* mice injected with mld-SZ + 0.25 mg/g L-NMMA.

The effect of MS from mice treated with citrate + PBS, mld-SZ + PBS, and mld-SZ + 0.25 mg/g L-NMMA on prestimulatory, net basal, and net and real stimulated secretion by dispersed rat islet cells was studied as an index of anti-β-cell CIA. Prestimulatory and net basal insulin secretion were not significantly different in any of the groups. Stimulated insulin secretion by dispersed rat islet cells was similar in the presence of MS from citrate + PBS mice or in the presence of only stimulatory medium (net stimulated insulin secretion, 31.87 ± 1.29 *v* 29.77 ± 0.20; real stimulated insulin secretion, 26.82 ± 1.29 *v* 24.61 ± 0.30 μU/5,000 cells/5 min; n = 5 and 6, respectively).

MS from mld-SZ + PBS and from mld-SZ + L-NMMA mice inhibited net and real stimulated insulin secretion

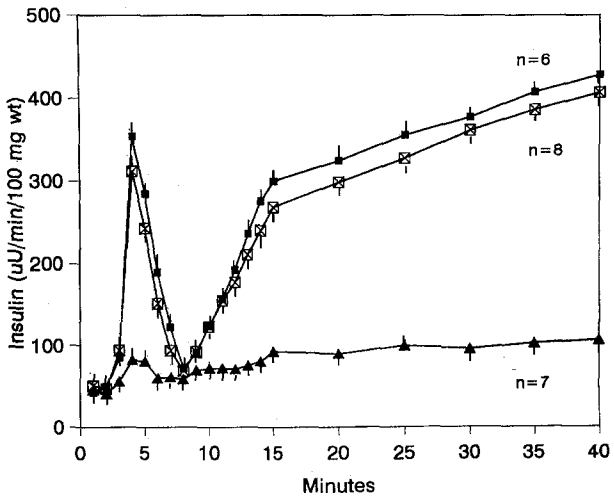

Fig 3. Insulin secretion patterns of perifused pancreatic slices from control (■), mld-SZ (▲), and mld-SZ + 0.25 mg/g L-NMMA mice (□). Results are the mean ± SEM.

compared with MS from citrate + PBS mice (Table 2). However, insulin secretion in the presence of MS from mld-SZ + L-NMMA mice was significantly higher than in the presence of MS from mld-SZ + PBS animals (Table 2).

L-NMMA in mld-SZ Mice: Effect on Transfer of MS to Recipient Mice

One injection of MS from mld-SZ mice to syngeneic normal recipients did not affect nonfasted serum glucose values, regardless of which treatment was applied to donor animals (Table 3).

The five groups of mice transferred with MS showed similar values for basal insulin secretion in perifused pancreatic islets (animals transferred with MS from mice injected with citrate + PBS, 0.15 ± 0.02 μU/min/islet, n = 6; mld-SZ + PBS, 0.19 ± 0.03, n = 5; mld-SZ + 0.17 mg/g L-NMMA, 0.22 ± 0.03, n = 5; mld-SZ + 0.25 mg/g L-NMMA, 0.21 ± 0.02, n = 6; and mld-SZ + 0.25 mg/g L-arginine, 0.25 ± 0.03, n = 6).

Mice transferred with MS from mld-SZ donors presented significant diminutions (41% to 46%) in first-phase glucose-induced insulin secretion. This was not observed when mice were transferred with MS from mld-SZ + 0.25 mg/g L-NMMA donors (Fig 4).

Second-phase glucose-stimulated insulin secretion showed significant diminution in all groups of mice transferred with MS from diabetic donors compared with mice transferred with MS from control donors (Fig 5).

Insulin secretion patterns of perifused pancreatic islets from mice injected with MS from control, mld-SZ, or mld-SZ + 0.25 mg/g L-NMMA donor mice are shown in Fig 6.

MS from mice transferred with splenocytes from syngeneic donors previously injected with mld-SZ + PBS significantly inhibited net and real stimulated insulin secretion by dispersed rat islet cells. This inhibition was not observed when mice were transferred with splenocytes from donors previously injected in vivo with mld-SZ + L-NMMA 0.25 mg/g (Table 4).

Table 3. Serum Glucose Levels (mg/dL) in Mice Injected With MS From mld-SZ–Treated Donors

Treatment	Pretransfer	15 Days Posttransfer
Citrate + PBS	171.3 ± 3.3, n = 6	158.0 ± 8.9, n = 6
mld-SZ + PBS	171.2 ± 6.6, n = 5	166.8 ± 6.5, n = 5
mld-SZ + L-NMMA 0.17 mg/g	151.7 ± 4.5, n = 5	161.7 ± 4.9, n = 5
mld-SZ + L-NMMA 0.25 mg/g	160.3 ± 5.3, n = 6	156.5 ± 3.8, n = 6
mld-SZ + L-Arg 0.25 mg/g	152.7 ± 2.3, n = 6	158.8 ± 3.4, n = 6

NOTE. P = NS in all cases.

DISCUSSION

Sufficient evidence has been disclosed to assume that in mld-SZ-treated mice, β-cell destruction occurs during nonspecific islet inflammation, involving toxic mediators such as highly reactive oxygen free radicals, cytokines (mainly IL-1), and NO.[1,2,4,20] One of the major toxins produced by macrophages and endothelial cells is NO.[1,4] NO is toxic to islet cells, even in the absence of other inflammatory mediators and at concentrations shown to be nonlytic for other cell types.[4,5]

It was reported[5,20,21] that IL-1 induces the formation of NO in islet β cells through the reaction catalyzed by NO synthase; consequently, the endocrine β cell becomes an important source of NO production.

If NO mediates β-cell death, inhibition of NO generation should prevent or delay the first steps in the cascade of cellular events that culminate in insulin-dependent diabetes mellitus. Analogs of L-arginine, in which one of the guanidino-nitrogens is either methylated (L-NMMA, L-N^G-monomethyl-arginine) or nitrosylated (NAME, L-N^G-nitro-arginine methyl ester), have been shown to competitively inhibit NO synthase.[1,7,8]

Previous data reported by Lukic et al[9] showed that L-NMMA treatment of mld-SZ-injected mice partially prevented hyperglycemia and reduced lymphocytic infiltration into the islets, suggesting that NO may also be involved in the development of insulitis. Furthermore, IP administration of NAME after mld-SZ treatment suppressed the development of hyperglycemia and also partially decreased NO production and islet cell lysis.[22]

Our results clearly support the above-mentioned reports, since L-NMMA treatment not only prevents the mld-SZ-induced hyperglycemia, but also partially prevents the impairment in both phases of glucose-stimulated insulin secretion from perifused pancreatic slices. L-NMMA injections were given only for 5 days after mld-SZ treatment was completed, to avoid any direct interactions with the β-cell toxin.

L-NMMA, as an arginine derivative, could also have metabolic effects on β cells. However, another recent study[23] and the results reported herein do not support such effects. Although these studies provide evidence suggesting that prevention of in vivo NO formation may partially block the onset of the disease, the mechanism(s) involved is unknown. Several alternatives deserve consideration: (1) blood flow,[24] (2) a significant decrease of the total vascular

Table 2. Effect of MS From C3H Mice on Net Basal and Net and Real Stimulated Insulin Secretion by Dispersed Rat Islet Cells

Treatment	Insulin Secretion (μU/5,000 cells/5 min)		
	Net Basal	Net Stimulated	Real Stimulated
Citrate + PBS	5.06 ± 0.08, n = 5	31.87 ± 1.29, n = 5	26.81 ± 1.29, n = 5
mld-SZ + PBS	5.46 ± 0.16, n = 5	8.04 ± 1.19, n = 5*	2.58 ± 1.04, n = 5*
mld-SZ + L-NMMA	5.35 ± 0.08, n = 5	25.26 ± 0.78, n = 5*†	19.91 ± 0.77, n = 5*†

*P < .01 v citrate + PBS.
†P < .01 v mld-SZ + PBS.

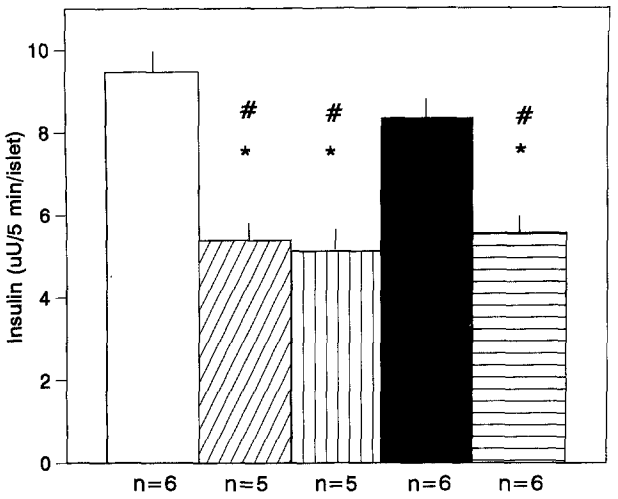

Fig 4. Areas under the first-phase glucose-stimulated insulin secretion in perifused pancreatic islets from mice transferred with 5 × 10^7 MS obtained from donors injected with citrate buffer + PBS (□), mld-SZ + PBS (▨), mld-SZ + 0.17 mg/g L-NMMA (▥), mld-SZ + 0.25 mg/g L-NMMA (■), and mld-SZ + 0.25 mg/g L-arginine (▤). Results are the mean ± SEM. *P < .01 v mice transferred with MS from citrate buffer–injected donors; #P < .01 v mice transferred with MS from mld-SZ + 0.25 mg/g L-NMMA–injected donors.

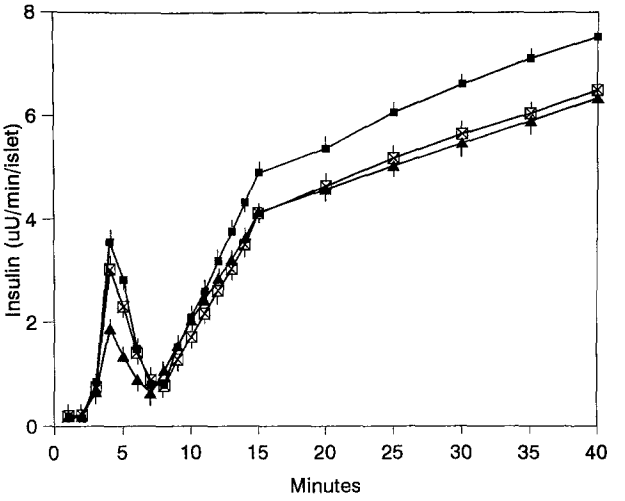

Fig 6. Insulin secretion patterns of perifused pancreatic islets from mice transferred with 5 × 10^7 MS obtained from control (■, n = 6), mld-SZ + PBS–injected (▲, n = 5), and mld-SZ + 0.25 mg/g L-NMMA–injected (□, n = 6) syngeneic donors. Results are the mean ± SEM.

bed and changes in vascular permeability,[25-28] (3) adhesion molecules,[29] and (4) the T-helper 1/T-helper 2 imbalance.[2]

Besides the endocrine pancreas, SZ also affects other organs, including the immune system. In addition to its cytotoxic activity,[30] NO appears to be a regulatory molecule of the immune system.[1,6-8]

The capacity of MS to inhibit stimulated insulin secretion

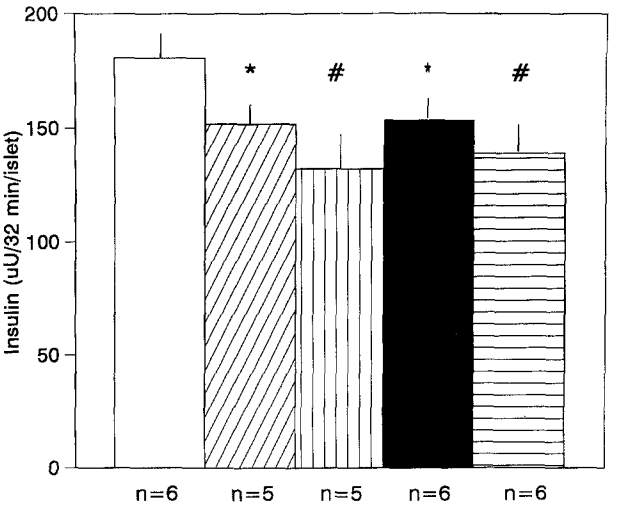

Fig 5. Areas under the second-phase glucose-stimulated insulin secretion in perifused pancreatic islets from mice transferred with 5 × 10^7 MS obtained from donors injected with citrate buffer + PBS (□), mld-SZ + PBS (▨), mld-SZ + 0.17 mg/g L-NMMA (▥), mld-SZ + 0.25 mg/g L-NMMA (■), and mld-SZ + 0.25 mg/g L-arginine (▤). Results are the mean ± SEM. *P < .05 and #P < .01 v mice transferred with MS from citrate buffer + PBS–injected donors.

by dispersed rat islet cells in vitro has been defined as a marker of anti–β-cell CIA.[17,31,32] Data from previous studies showed that peripheral blood mononuclear cells from newly diagnosed and chronically insulin-dependent diabetic patients developed CIA toward pancreatic β cells.[18] The inhibitory effects of mononuclear cells appear to be specific for pancreatic β cells, since no cytotoxicity is observed against fibroblasts; furthermore, secretion of glucagon[17,31] and somatostatin[18] remains unaffected. Such inhibition of insulin secretion is not altered by insulin treatment or the presence of antibody, complement, or aggregated IgG.[17,31,32] Cytoadherence of diabetic lymphocytes is increased in two xenogeneic species (rat and hamster), but not in seven non–insulin-secreting cell lines.[32,33]

Data from our studies showed that MS from mld-SZ mice developed CIA toward pancreatic β cells. In vivo L-NMMA administration partially prevented CIA. Thus, inhibition of NO synthase activity could prevent islet infiltration by affecting early steps of the immune reaction.

Recently, strong NO synthase expression determined at the level of transcription, translation, and enzymatic activity was associated with destructive insulitis in NOD mice.[34] Furthermore, the kinetics of NO synthase expression correlates with that of IFN-γ, both at mRNA and protein levels.

The close correlation between NO synthase and IFN-γ expression and insulitis development supports the hypothesis that intraislet NO production is involved in β-cell destruction.[34]

In irradiated NOD mice, administration of aminoguanidine, another inhibitor of NO production, delayed the onset of diabetes after spleen cell transfer in receptor mice. However, this protective in vivo effect of aminoguanidine was small and highly dependent on the amount of cells transferred, and seemed to act mainly on the receptor pancreas.[35]

Table 4. Effect of MS From Mice Transferred With MS From Different Syngeneic Donors on Net Basal and Net and Real Stimulated Insulin Secretion by Dispersed Rat Islet Cells

Treatment	Insulin Secretion (μU/5,000 cells/5 min)		
	Net Basal	Net Stimulated	Real Stimulated
Citrate + PBS	5.24 ± 0.06, n = 6	29.75 ± 0.19, n = 6*	24.50 ± 0.21, n = 6*
mld-SZ + PBS	5.22 ± 0.06, n = 4	17.57 ± 1.25, n = 4	12.35 ± 1.28, n = 4
mld-SZ + L-NMMA	5.18 ± 0.06, n = 6	27.84 ± 1.17, n = 6*	22.66 ± 1.17, n = 6*

*$P < .01$ *v* mld-SZ + PBS.

As previously reported, normal mice transferred with MS from mld-SZ syngeneic donors show an impairment in the pattern of in vitro glucose-stimulated insulin secretion[12] and also develop CIA. Data from the present study indicate that in vivo L-NMMA administration to diabetic donor mice prevents this inhibition of in vitro first-phase insulin secretion and CIA toward rat pancreatic β cells in recipient mice.

Although a nonimmunological mechanism(s) of diabetes prevention by L-NMMA cannot be excluded, these results suggest that L-NMMA treatment could also act on T-cell–dependent immune reactions.

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