Insulin Secretion by Pancreas of Athymic Mice Injected With Peripheral Mononuclear Cells From Insulin-Dependent Diabetic Patients

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We studied the effect of peripheral blood mononuclear cells (PBMNC) from insulin-dependent diabetic (IDDM) children on the insulin secretion pattern of the pancreas from recipient athymic mice. PBMNC from healthy controls or IDDM patients in different stages of disease were injected into athymic mice. PBMNC from newly diagnosed IDDM children elicited basal nonfasting hyperglycemia and in vitro inhibition of the first and second phases of glucose-stimulated insulin secretion in recipient mice. Animals injected with cells from chronically IDDM children showed normoglycemia, abnormal tolerance to glucose, and inhibition of first-phase insulin secretion. Mitomycin C treatment of MNC from IDDM patients abolished insulin secretion inhibition in recipient mice. PBMNC from newly diagnosed and chronically IDDM patients showed positive anti–β-cell cellular immune aggression. Mice injected with cells from patients during the remission period showed normoglycemia and no alteration of insulin secretion patterns. When relapsed to their former clinical stage, injection of the cells significantly inhibited first-phase glucose-induced insulin secretion in recipients. PBMNC from newly diagnosed IDDM patients were found to migrate to the pancreas of recipient mice preferably as compared with cells from controls. Cells from chronically IDDM patients cultured with concanavalin A (Con A) increased insulin secretion inhibition; despite this, cells from children during the remission period cultured with Con A failed to modify insulin secretion in recipients. These results show that injection of PBMNC from diabetic patients leads to insulin secretion impairment in recipient mice pancreas, and provide a basis for the study of mechanisms involved in the onset and modulation of anti–β-cell cellular immune aggression induced by human PBMNC.

DURING THE LAST two decades, enough evidence has been provided to conclude that insulin-dependent diabetes mellitus (IDDM) is brought about by an immune process.[1-3]

Transfer experiments from diabetic- to non–diabetic-prone animals demonstrated that T cells mediated the anti–β-cell immune aggression.[4] This was also supported by the capacity of purified T lymphocytes to inhibit insulin release from islet cells in vitro.[5,6] Buschard et al[7] reported that passive transfer of peripheral blood mononuclear cells (PBMNC) from newly diagnosed IDDM patients elicited hyperglycemia in athymic mice.

In the present report, we study the effect of injection of PBMNC from IDDM patients in several evolutive stages of disease into athymic mice. The evolution of glycemia levels and the insulin secretion patterns were investigated in recipient mice. To evaluate the role of PBMNC in the development of pancreatic alterations, we examined the trapping of PBMNC by recipient mice pancreas and the anti–β-cell cellular immune aggression (defined as the ability of PBMNC to inhibit insulin secretion when cultured in vitro with dispersed rat islet cells).

SUBJECTS AND METHODS

Patient Population

Parental written consent was required for all subjects participating in this study. The protocol was approved by the Ethics Committee of the Hospital de Niños, Buenos Aires, Argentina.

The following groups of children were studied. (1) Healthy control group: 18 healthy children (10 boys and eight girls, aged 6 to 15 years) free of autoimmune or endocrine disease and with no family history of diabetes. Fasting glycemia values were 85.62 ± 2.18 mg/dL. (2) IDDM children who were assigned to three different evolutive groups: newly diagnosed, 25 children (12 boys and 13 girls aged 6 to 13 years), eight before insulin therapy and 17 with up to 1 month of insulin therapy, free of associated pathology. All had fasting glycemia values greater than 250 mg/dL at diagnosis. Five newly diagnosed IDDM children with no previous insulin therapy were also studied 25 to 30 days and 8 to 10 months after insulin treatment; long-standing, 34 children (14 boys and 20 girls aged 5 to 14 years) with 4 to 31.4 months of insulin therapy, and free of other associated pathology; and remission, 13 children (five boys and eight girls, aged 6 to 16 years) free of associated pathology. Clinical remission was defined as the situation in which patients remained aglycosuric and aketonuric, with fasting glycemia values less than 120 mg/dL, for at least 15 days after insulin injection withdrawal.

Mice

Male and female nude mice (8 weeks old) of the BALB/c (nu/nu and +/nu) strain were used as PBMNC recipients. The animals were provided by a local colony from the Departamento de Radiobiología, Comisión Nacional de Energía Atómica (CNEA), Buenos Aires, Argentina. They were kept five per cage under sterile conditions. Sterile normal feeding pellets and drinking water were supplied ad libitum. The animals were maintained in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals.*

PBMNC Transfer Procedure

This was performed according to the method reported by Buschard et al.[7] Children underwent 12 hours of fasting. Blood extraction (20 mL venous blood into a heparinized syringe) was performed before insulin administration on the day of study.

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Submitted September 10, 1994; accepted March 14, 1995.

Supported by grants from Fondo Cruz del Sur, Fundación A.J. Roemmers, and CONICET, Argentina.

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0026-0495/95/4411-0012$03.00/0

Plasma was separated by centrifugation in a Ficoll-Hypaque gradient (Ficollpaque; Pharmacia, Uppsala, Sweden). PBMNC were collected in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 μg/mL streptomycin, and 100 U/mL penicillin (Gibco, Grand Island, NY). Cellular viability was assessed with the Trypan test,[8] and PBMNC concentration was adjusted to 1.2×10^7 viable cells/mL. PBMNC from each individual were injected intraperitoneally using sterile syringes and needles into five mice. Each mouse received 6×10^6 cells in 0.5 mL medium.

PBMNC Modifications Before Transfer

In another group, cells were incubated 72 hours with concanavalin A (Con A; Sigma, St Louis, MO) 10 μg/10^6 cells/mL in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 μg/mL streptomycin, and 100 U/mL penicillin at 37°C in a humidified atmosphere containing 95% air and 5% CO_2. After incubation, the cells were washed three times with fresh medium and the concentration was adjusted to 1.2×10^7 cells/mL. Each mouse received intraperitoneally 6.0×10^6 cells in 0.5 mL medium. In another group, PBMNC were incubated 20 minutes at 37°C with mitomycin C (Sigma) 1 μg/1.0×10^6 cells/mL in RPMI 1640 medium supplemented and washed as previously indicated, and the concentration was adjusted to 1.2×10^7 viable cells/mL. Each mouse received intraperitoneally 6×10^6 viable cells in 0.5 mL medium.

Mice Blood Glucose Determination

Blood of nonfasting mice was collected from the paraorbital venous plexus before and 8, 16, and 24 days after PBMNC injection. Blood glucose level was determined in a Beckman Spectrophotometer DBG (Munich, Germany), using a Glycemia enzymatic kit (Weiner, Buenos Aires, Argentina).

Perifusion of Pancreatic Slices

The technique described by Burr et al[9] was used. Krebs-Ringer bicarbonate supplemented with 1% bovine albumin fraction V (Sigma) and 3.3 mmol/L glucose was used as perifusion buffer. The pH of the buffer (kept under constant 95% O_2 and 5% CO_2 gassing) was 7.38 to 7.40. Perifusion flux was 1.8 to 2.2 mL/min. The proteolytic effect on hormone secretion during perifusion was avoided by adding Trasylol 1,000 KIU/mL (Bayer, Buenos Aires, Argentina) to the perifusion buffer and collecting the samples on 0.25 mol/L EDTA at 4°C. Samples were immediately frozen at −20°C. Slices from the whole pancreas of a single mouse were used in each perifusion. Samples were collected following an initial 15-minute recovery period. Perifusion samples from minutes 0 and 1 were used for baseline determinations. A stimulus of 16.5 mmol/L glucose was added between minutes 2 and 40; first-phase insulin secretion was measured between 4 and 8 minutes, and the second phase between 10 and 40 minutes. Samples were kept at −20°C until insulin determination by radioimmunoassay.

Study of Insulin Secretion by Dispersed Rat Islet Cells Incubated With Human PBMNC (cellular immune aggression)

These studies were performed simultaneously with the transfer. PBMNC were processed immediately after blood collection obtaining a PBMNC-rich preparation from a 15-mL sample by means of a Ficoll-Hypaque gradient. Cells were washed twice with Hanks buffer and suspended in Minimal Essential Medium ([MEM] Gibco, Paisley, UK) modified as described later, to reach a final concentration of 4×10^6 MNC/mL.

Islets of Langerhans were obtained from collagenase-treated (Serva Feinbiochem, Heidelberg, Germany) adult Wistar rat pancreas (CNEA) according to the method reported by Lacy and Kostianovsky.[10] To obtain islet cell suspensions, freshly isolated islets were treated with EDTA and trypsin (Sigma) as described by Ono et al.[11]

Islet cells were suspended in basal MEM (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[5] 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 PBMNC from either control subjects or IDDM patients (4×10^5 cells per well in 100 μL) or basal medium (100 μL), and incubated for 18 hours. Ten wells were used for each IDDM patient or control PBMNC preparation. 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 by 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); 16.5 mmol/L glucose alone also stimulated insulin secretion (0.202 ± 0.018 pmol/5,000 cells/5 min, n = 10). Five wells for each basal or stimulatory medium were used. PBMNC remained with the islet cells during both basal and stimulatory periods. Supernatants were withdrawn after 5 minutes and rapidly frozen for insulin determination.

Net basal secretion is presented as the insulin secretion (picomoles per 5,000 cells) during a 5-minute incubation period in the presence of basal medium minus 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.

Results are expressed as real stimulated insulin secretion or as the insulin secretion index: insulin secretion index = [(stimulated release − basal release)/basal release] × 100.

The reproducibility of cellular immune aggression (CIA) test results (real stimulated secretion) had a 4.01% interassay coefficient of variation (CV) and a 4.23% intraassay CV.

PBMNC Trapping by Athymic Recipient Mice

Venous blood was collected from control and IDDM patients, and PBMNC were isolated as described earlier. Erythrocytes were lysed using 0.83% NH_4Cl (Mallinkrodt, Chem Works, St Louis, MO) for 8 minutes at 37°C. PBMNC were washed three times with RPMI 1640 medium supplemented with 10% fetal calf serum, 100 mg/mL streptomycin, and 100 U/mL penicillin and incubated with $Na_2{}^{51}CrO_4$ (CNEA) 25 μCi/10^8 cells/mL for 30 minutes at 37°C in a humidified atmosphere (95% air/5% CO_2). After incubation, PBMNC were washed using fresh medium, and the concentration was adjusted to 1×10^8 viable cells/mL.

Ten aliquots of viable resuspended cells were incubated for 12, 24, or 48 hours to observe spontaneous ^{51}chromium release. The percent of spontaneous release at the cited times was 0.70 ± 0.06, 1.02 ± 0.09, and 1.72 ± 0.26, respectively.

Each mouse (five for each control or IDDM group) received 1×10^9 cells intraperitoneally in 0.1 mL medium. Recipient mice were killed 24 hours after injection, their organs were removed, and ^{51}Cr activity was measured in blood cells (obtained from 0.1 mL whole blood), plasma (0.1 mL), one kidney, whole liver, whole spleen, one lung, whole pancreas, and lymph modes (mesenteric, inguinal, and axillary). A group of PBMNC from IDDM patients were labeled

with $Na_2{}^{51}CrO_4$ and lysed by freeze-thawing in distilled water. After centrifugation, 0.2 mL of the supernatant was injected into each athymic mice and treated as described earlier. Results were expressed for each organ as percent ^{51}Cr uptake = (cpm per organ/total cpm) × 100, where total cpm = cpm in all organs tested in each mouse.

Insulin Radioimmunoassay

Insulin was determined by radioimmunoassay[12] using purified rat insulin as standard (Novo Nordisk, Gentofte, Denmark), porcine ^{125}I-insulin as tracer (CNEA), and a guinea pig anti-porcine insulin antiserum. The intraassay CV 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 CV was 6.6%, 5.9%, and 5.2%, respectively, for the given ranges.

Statistical Analysis

In all cases, results are expressed as the mean ± SEM. The areas under insulin secretion curves were integrated to evaluate insulin secretion in perifusion of pancreas slices. Statistical analysis of the data was performed with two-tailed Student's *t* test for unpaired samples. ANOVA[13] and Scheffe's test for multiple comparisons between individual groups were used. *P* less than .05 was considered statistically significant.

RESULTS

Evolutive Glycemia Values in Recipient Mice Injected With PBMNC From Control and IDDM Children

Recipient mice injected with PBMNC from controls, chronically IDDM children, and IDDM children during a remission period showed normoglycemia until the end of the study (Table 1). Mice injected with PBMNC from newly diagnosed IDDM patients showed a significant increase in nonfasted glycemia values ($P < .001$) at days 8, 16, and 24 after PBMNC injection.

Insulin Secretion Patterns of PBMNC Recipient Mice

Injection of PBMNC from newly diagnosed IDDM patients induced a diminished first-phase ($P < .001$) and second-phase ($P < .01$) glucose-stimulated insulin secretion from recipient mice pancreas (Table 2 groups 1 and 2, and Fig 1).

There was no difference in the amount of insulin inhibited when injected PBMNC came from children previously treated or untreated with insulin (first-phase insulin secretion, 6.54 ± 0.29 *v* 6.19 ± 0.33 pmol/5 min/100 mg; second-phase, 85.44 ± 2.02 *v* 82.64 ± 2.42 pmol/30 min/100 mg; n = 17 and n = 8, respectively). The insulin secretion pattern of five IDDM children was studied in different stages of the disease: (1) newly diagnosed before insulin therapy (first-phase, 6.80 ± 0.59 pmol/5 min/100 mg, n = 10; second-phase, 85.88 ± 2.28 pmol/30 min/100 mg, n = 10), (2) after 25 to 30 days of insulin therapy (first-phase, 6.31 ± 0.39, n = 10; second-phase, 86.15 ± 2.19, n = 10), and (3) after 8 to 10 months of insulin therapy (first-phase, 7.95 ± 0.48, n = 9; second-phase, 117.19 ± 2.79, n = 9). In all cases, n represents the number of perifusions from separated mice.

Recipient mice injected with PBMNC from chronic diabetics showed a significantly diminished first-phase glucose-stimulated insulin secretion (Table 2 group 3, and Fig 1).

Four patients were evaluated in three different stages of the disease: before, during, and after the remission period. They were all included in the long-standing IDDM group, since their remission appeared approximately 1 year after diagnosis (7 to 13 months after diagnosis). During remission, PBMNC transfer did not alter insulin secretion patterns in recipient mice. When patients relapsed to their former stages, PBMNC transfer significantly inhibited first-phase glucose-stimulated insulin secretion in recipient mice ($P < .01$) (Table 2 groups 4 to 7).

Effect of PBMNC Culture With Con A Before Transfer

PBMNC from controls, chronically IDDM patients, and IDDM patients in remission were incubated with the mitogen Con A before injection to modulate insulin secretion inhibition in recipient mice. Control PBMNC cultured with Con A did not alter insulin secretion patterns in recipient mice. PBMNC from chronically IDDM patients cultured with Con A significantly increased first-phase insulin secretion inhibition in mice ($P < .01$). Remission PBMNC cultured with Con A failed to alter insulin secretion patterns in mice (Table 2 groups 8 to 10).

Effect of PBMNC Incubation With Mitomycin C Before Transfer

PBMNC from some IDDM patients that alter insulin secretion patterns in mice (ie, long-standing and newly diagnosed patients) were incubated with mitomycin C before transfer. When incubated with mitomycin C, they did not inhibit insulin secretion in recipient mice (Table 2 groups 11 to 13).

In Vitro Inhibition of Insulin Secreted by Dispersed Rat Islet Cells Incubated With Human PBMNC (CIA)

Table 3 shows that PBMNC from IDDM children (both newly diagnosed and long-standing) significantly inhibited

Table 1. Nonfasted Glycemia Values in BALB/c (nu/nu) Mice Injected With 6 × 10^6 PBMNC From Control or IDDM Children

PBMNC Donor	Initial	Days Postinjection		
		8	16	24
Control (n = 18)	145.3 ± 4.8	143.7 ± 5.7	138.7 ± 5.5	139.8 ± 6.7
Newly diagnosed (n = 25)	136.7 ± 4.8	182.9 ± 6.3*	190.0 ± 10.0*	195.0 ± 11.1*
Long-standing (n = 34)	132.8 ± 4.9	126.5 ± 5.7	122.0 ± 3.4	130.5 ± 5.0
Remission (n = 13)	125 ± 2.5	122.3 ± 4.2	138.2 ± 5.8	121.8 ± 5.9

NOTE. Results are the mean ± SEM; n = number of children tested.

*$P < .001$ *v* initial value.

Table 2. Insulin Secretion by Pancreas of Recipient Athymic Mice Injected with PBMNC From Controls and IDDM Patients

Source of PBMNC Injected in Recipient Mice	Insulin Secretion First-Phase (pmol/4 min/100 mg)	Insulin Secretion Second-Phase (pmol/30 min/100 mg)	No.
1. Control	11.47 ± 0.47	121.46 ± 1.99	12
2. ND IDDM	6.36 ± 0.19*	84.02 ± 2.29†	16
3. LS IDDM	8.87 ± 0.44†	117.72 ± 2.21	12
4. Control	10.76 ± 0.43	120.63 ± 1.91	4
5. LS IDDM (before remission)	8.14 ± 0.16†	117.80 ± 2.76	4
6. LS IDDM (during remission)	10.42 ± 0.55	121.41 ± 2.42	4
7. LS IDDM (after remission)	8.32 ± 0.42†	123.88 ± 2.27	4
8. LS IDDM	8.07 ± 0.26†	119.72 ± 2.99	12
9. LS (Con A culture)	5.52 ± 0.39*‡	103.60 ± 2.09†‡	12
10. LS IDDM during remission (Con A culture)	11.02 ± 0.77	117.99 ± 2.85	10
11. Control	10.27 ± 0.45	121.53 ± 1.86	6
12. ND IDDM (mitomycin C-treated)	9.97 ± 0.61	119.54 ± 2.55	6
13. LS IDDM (mitomycin C-treated)	10.86 ± 0.35	123.42 ± 2.47	6

NOTE. Number of separated mouse perifused pancreas: groups 1 to 3, 25 for each one; groups 4 to 7 and 11 to 13, 10 for each one; and groups 8 to 10, 15 for each one. Results are the mean ± SEM.

Abbreviations: ND, newly diagnosed; LS, long-standing.

*$P < .001$ *v* controls.

†$P < .01$ *v* controls.

‡$P < .01$ *v* LS IDDM.

the real insulin secretion stimulated by glucose-theophylline ($P < .001$). Table 3 also shows that real insulin secretion in the presence of PBMNC from six IDDM children during remission was no different from controls.

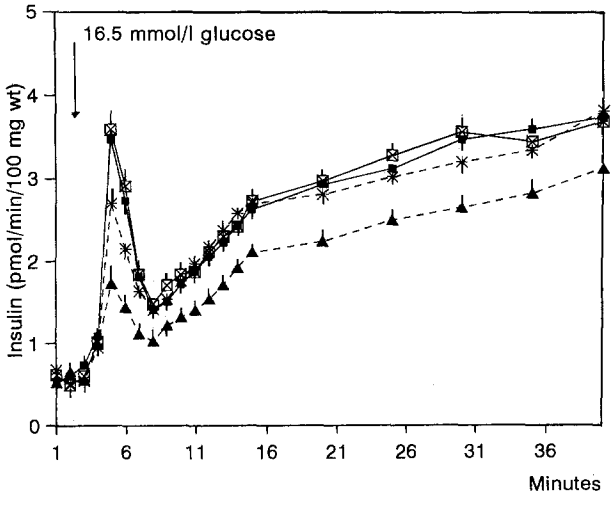

Fig 1. Patterns of insulin secretion by perifused pancreatic slices from mice injected with PBMNC of IDDM children (▲, newly diagnosed, n = 16; *, long-standing, n = 12; ☒, remission, n = 8; and ■, controls, n = 8). Results are the mean ± SEM. Stimulus, 16.5 mmol/L glucose. n, number of children tested. Number of separated mouse perifused pancreas, 25 for each group.

Table 3. Real Stimulated Insulin Secretion by Dispersed Rat Islet Cells Incubated in the Presence of PBMNC From Controls and IDDM Patients (considered an index of CIA)

PBMNC Donor Group	Insulin Secretion (pmol/5 × 10^3 cells/5 min)
Control (n = 15)	0.26 ± 0.02
Newly diagnosed IDDM (n = 9)	0.04 ± 0.02*
Long-standing IDDM (n = 13)	0.10 ± 0.03*
Long-standing IDDM during remission (n = 6)	0.21 ± 0.04

NOTE. Results are the mean ± SEM. Stimulus, glucose 16.5 mmol/L plus theophylline 5 mmol/L; n, number of children tested.

*$P < .001$ *v* control.

Net basal insulin secretion was no different in any of the groups studied.

In Fig 2, individual results are expressed as the insulin secretion index.

PBMNC Trapping by Recipient Mice Pancreas

Table 4 shows pancreatic trapping of PBMNC from controls and newly diagnosed IDDM patients 24 hours after cell injection. ^{51}Cr uptake was significantly increased in the pancreas of recipient mice injected with PBMNC from IDDM children ($P < .001$). There was no difference in the remaining organ uptake among mice injected with control or IDDM PBMNC. Differences observed in the pattern of ^{51}Cr uptake when lysed PBMNC were used indicate that ^{51}Cr activity in the other two groups was

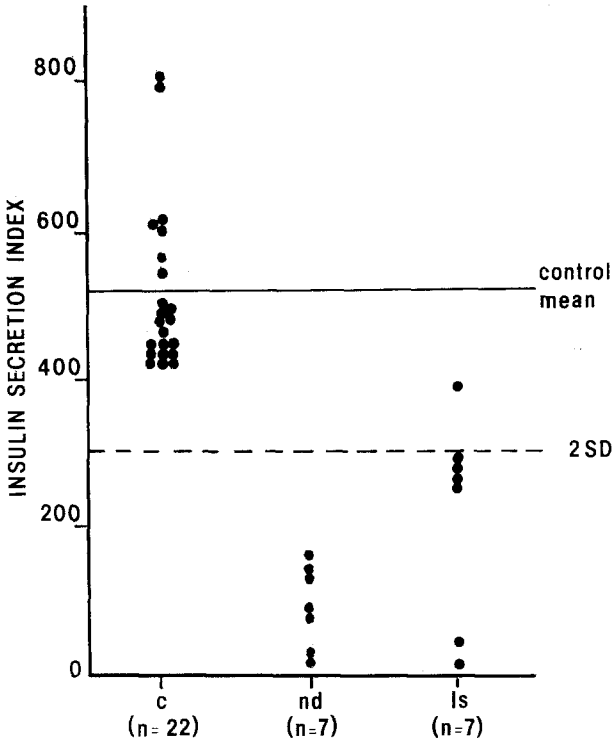

Fig 2. Effect of PBMNC from controls (c), newly diagnosed (nd), and long-standing (ls) IDDM patients on insulin secretion index by dispersed rat islet cells (CIA). Results are expressed as the mean ± SEM.

Table 4. Trapping of $Na_2{}^{51}CrO_4$ by Different Organs of Athymic Mice Injected With Labeled PBMNC From Controls and Newly Diagnosed IDDM Patients

Organ	Lysated PBMNC Supernatant (n = 4)*	Injected With PBMNC From: Controls (n = 6)	Injected With PBMNC From: Newly Diagnosed IDDM Patients (n = 6)
Cell blood	0.27 ± 0.05	2.61 ± 0.47	2.54 ± 0.95
Plasma (0.1 mL)	4.50 ± 0.21	2.51 ± 0.38	2.54 ± 0.95
Liver	58.65 ± 6.00	62.34 ± 2.86	60.00 ± 3.77
Pancreas	1.39 ± 0.27	5.00 ± 0.40	8.32 ± 0.38†
Kidney	27.55 ± 2.51	7.36 ± 0.90	6.03 ± 0.59
Spleen	2.13 ± 0.26	14.47 ± 0.94	15.01 ± 1.06
Lung	4.88 ± 0.15	3.18 ± 0.57	3.25 ± 0.86
Lymph nodes	0.63 ± 0.06	2.53 ± 0.14	2.31 ± 0.08

NOTE. Results are the mean ± SEM. Number of recipient mice injected: 20, 30, and 30 for lysate, control, and diabetic groups, respectively.

*$P < .001$ for all organ uptake *v* the other 2 groups.

†$P < .001$ *v* control.

carried by nondamaged viable cells. Recipient mice showed no difference in pancreatic trapping of PBMNC from long-standing IDDM children as compared with controls (control 5.56% ± 0.34% *v* IDDM 5.25% ± 0.37%, n = 4 for both groups). However, when PBMNC from long-standing IDDM patients were previously incubated with Con A, the recipients' pancreatic trapping increased (7.95% ± 0.58%, n = 4, $P < .001$) as compared with the control group.

DISCUSSION

This study shows that transfer of PBMNC from newly diagnosed and long-standing IDDM children to athymic mice led to an impairment of insulin secretion by the recipient pancreas. When PBMNC donors are newly diagnosed IDDM patients, the inhibition of insulin secretion by recipient mice is greater and hyperglycemia is present. These results are in agreement with Buschard et al,[7] who showed that passive transfer of PBMNC from newly diagnosed IDDM patients elicited hyperglycemia in BALB/c (nu/nu) recipient mice. On the other hand, several studies could not reproduce these results.[14-16] In 1989, we reported preliminary results showing the inhibitory effect of PBMNC from diabetic children on insulin secretion by athymic mice pancreas.[17] Recently, it has been demonstrated that PBMNC, and not plasma, from six of 10 newly diagnosed patients induce insulitis in the pancreas of recipient athymic mice.[18]

We have to point out that the above-cited studies, whether they succeeded in producing hyperglycemia, did not evaluate insulin secretion in recipient mice. In our experimental conditions, insulin secretion inhibition was a sensitive index of β-cell function impairment.

The xenogenicity of our transfer procedure, in which human PBMNC seem to recognize and affect mice islet cells, raises the problem of the apparent absence of MHC restriction. An "inflammatory model" in which the transfer of PBMNC provides antigen-presenting cells that process an islet-associated human/murine cross-reactive antigen in the correct MHC context (human class II antigens), to an islet-reactive CD4 T cell from the donor, has been postulated.[18] Recently, Gelber et al[19] showed that NOD T-cell proliferative responses to a murine insulinoma–fractionated β-cell antigen were also observed to a fractionated extract of human islets. This suggests potential shared antigenic determinants between human and mouse β cells.

Previous studies also showed that the disease process in spontaneously diabetic animals could be the result of a CD4 T-cell–dependent inflammatory damage, and that islet cell disruption could be mediated by cytokines and oxygen radicals, both toxic to xenogeneic tissues.[20,21] Subsets of T cells expressing CD3-associated receptor composed of δ and γ chains, double negative (CD4−, CD8−), had been implicated in a variety of autoimmune conditions.[22] Double-negative T cells had also been found among islet-inflammatory T cells in human IDDM.[23] Therefore, a role for this double-negative T cell in the impairment of insulin secretion cannot be ruled out.

Previous studies from Logothetopoulos et al[24] suggest that donor PBMNC undergo a phase of proliferation in lymphoid organs of recipients before initiating the autoimmune process in the pancreatic islet of the BB rat. When transferring splenocytes from multiple low-dose streptozotocin–induced diabetic mice to normal recipients, we found some evidence to suggest that proliferation of injected cells enhanced progression to the diabetic state, and that both donor and recipient T lymphocytes played an important part in this progression.[25] Our results do not clarify whether the transferred cells are activated in the mouse or only continue the immune aggression for which they have been triggered in the donor IDDM children.

The capacity of PBMNC to inhibit stimulated insulin secretion by dispersed rat islet cells in vitro has been defined as a marker of anti–β-cell CIA.[5,6,26] Data from our studies showed that PBMNC from newly diagnosed and long-standing IDDM patients developed CIA toward pancreatic β cells.

Inhibitory PBMNC effects appear to be specific for pancreatic β cells, since no cytotoxicity is observed against fibroblasts; furthermore, secretion of glucagon[5,6] and somatostatin (control splenocytes, 19.12 ± 0.77; diabetic splenocytes, 18.27 ± 0.69 pg/5 × 10^3 cells/5 min; n = 4 in both cases) remains unaffected during the test. Such insulin secretion inhibition is not altered by insulin treatment or the presence of antibody, complement, or aggregated IgG.[5,6,27] Binding to RIN cells or islets by PBMNC from IDDM patients, but not from nondiabetics with other autoimmune diseases, can be observed.[18] Moreover, cytoadherence of diabetic lymphocytes increased in two xenogeneic species (rat and hamster), but not in seven non–insulin-secreting cell lines.[27,28]

Mitomycin C treatment of IDDM PBMNC before injection abrogated their effect on recipient insulin secretion. Whether this effect is due to an impairment of cytokines and/or oxygen radical production deserves further study. Furthermore, PBMNC from newly diagnosed and long-standing (previously cultured with Con A) IDDM patients showed a specific homing toward the pancreas, which might

indicate an incipient immune aggression phenomenon. The role of adhesion molecules in adoptive transfer in NOD mice has been reported.[29] These molecules, besides allowing cell contact, could also provide costimulatory signals for T-lymphocyte activation.

The combined results from in vitro CIA, mitomycin C treatment, and pancreatic trapping seem to support the assumption that the insulin secretion impairment observed was caused by PBMNC injection. It could be hypothesized that these results reflect an antiinsulin immunity resulting from insulin treatment rather than CIA. However, insulin secretion is impaired in newly diagnosed IDDM children before insulin treatment, and furthermore, the temporal study of five IDDM children also shows impairment of insulin secretion before and after insulin therapy. In a previous study, we found that a group of type II diabetic patients with secondary failure to oral hypoglycemic agents presented autoimmunity (CIA+) toward pancreatic β cells. However, there was no constant correlation between the presence of CIA and antiinsulin antibodies or insulin therapy.[30]

PBMNC from both newly diagnosed and long-standing IDDM patients inhibited insulin secretion in recipient mice. In the latter, the insulin secretion alteration seems insufficient to cause basal hyperglycemia. Incubation of long-standing IDDM PBMNC with the polyclonal mitogen Con A before injection increased the degree of insulin secretion inhibition in recipient mice and also produced hyperglycemia.

Insulin secretion from recipient mice pancreas was normal when transferred with PBMNC from IDDM patients during remission, even after Con A treatment. However, the ability of PBMNC to inhibit insulin secretion reappeared after remission, suggesting that other mechanisms different from a single effector-cell dilution could be responsible. Furthermore, PBMNC from IDDM children during remission did not show CIA, since they did not inhibit the real insulin secretion by dispersed rat islet cells (CIA, immunologic remission?). Buschard et al[31] reported that lymphocyte suppressor function was diminished in diabetes at diagnosis, and that this activity was normal during the remission period. Also, Charles et al[32] described an increase in cytotoxic lymphocyte function at diagnosis and a normalization at remission. Despite these facts, we cannot assume that remission in diabetes is caused by a normalization in lymphocyte subset function. It is well known that lymphocytes have hormonal and metabolic modulation.[31] During remission, an improved hormonal and metabolic medium influence on lymphocyte activities should be taken into consideration.

In conclusion, our results show that injection of PBMNC from IDDM patients induces an impairment of insulin secretion from the pancreas of recipient athymic mice. Temporal changes in the capacity of PBMNC to inhibit insulin secretion were also observed. This experimental model is an attractive tool to study mechanisms of β-cell function impairments induced by human immune cells.

ACKNOWLEDGMENT

We gratefully acknowledge the technical assistance of Susana Santillo, Miguel Garramone, and Pablo Díaz, and the secretarial help of Graciela Collazo and María Mones. Trasylol was a generous gift from Bayer, Buenos Aires, Argentina.

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