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Role of infiltrating T cells for impaired glucose metabolism in pancreatic islets isolated from non-obese diabetic mice

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Summary. Pancreatic islets isolated from non-obese diabetic (NOD) mice, all of which have insulitis, exhibit an impaired glucose metabolism. In order to investigate the role of infiltrating lymphocytes for this altered metabolism, we injected 12- to 13-week-old female NOD mice with monoclonal antibodies directed against either the $\alpha\beta$-T cell receptor, $CD4^+$ or $CD8^+$ T cells. Control NOD mice were injected with normal rat IgG or with the vehicle (phosphate buffered saline) alone. Injection of the three different monoclonal antibodies markedly reduced the mononuclear cell infiltration. An intravenous glucose tolerance test showed no differences between the groups. Islet insulin release in response to glucose was similar in all groups. In contrast, islets isolated from the control NOD mice with insulitis showed a high basal (1.7 mmol/l glucose) glucose oxidation rate and a small increase in the glucose oxidation rate in response to a high glucose concentration (16.7 mmol/l glucose). The monoclonal antibodies counteracted the elevated basal glucose oxidation rate of the islets. Parallel studies of stimulated mononuclear cells suggested that the contribution of glucose oxidized by islet-infiltrating lymphocytes could only partially explain the observed alterations in NOD mouse islet metabolism. Culture of islets obtained from NOD mice in the presence of the cytokine interleukin-1 β induced a similar pattern of glucose metabolism as seen earlier in IgG or phosphate-buffered saline treated control NOD mice. In conclusion, alterations in the glucose oxidation rates seem to be an early sign of disturbance in islets isolated from NOD mice. These early alterations in glucose metabolism can be reversed in vivo by monoclonal antibodies directed against effector lymphocytes. This suggests that the infiltrating mononuclear cells can induce reversible alterations in pancreatic Beta-cell function which may precede impaired insulin secretion, Beta-cell destruction and overt diabetes mellitus.

Key words: Non-obese diabetic mice, diabetes mellitus, insulin release, glucose oxidation, pancreatic islets, T lymphocytes.

The non-obese diabetic (NOD) mouse spontaneously develops Type 1 (insulin-dependent) diabetes mellitus which strongly resembles the human disease [1, 2]. The clinical disease is usually observed after 10 weeks of age, predominantly in females. The selective destruction of insulin-secreting Beta cells is preceded by an early infiltration of the islets of Langerhans by mononuclear cells, mainly T cells. Macrophages have been characterized in the early stages of insulitis [3, 4]. $CD4^+$ T cells are initially predominant among the T cells [5, 6] while $CD8^+$ T cells are secondarily recruited [7, 8]. Several reports suggest that $CD4^+$ T cells are central to the autoimmune activation process in the NOD mouse model. Cytotoxic $CD8^+$ T cells have been defined as possibly mediating Beta-cell destruction both in vitro [9, 10] and in vivo [11–13]. However, other data based on studies both in the NOD mouse [14] and in man [15] suggest that $CD4^+$ T cells may also act as effector cells.

Islets isolated from female 12- to 13-week-old NOD mice have previously been shown to exhibit an impaired glucose metabolism [16]. Since these islets all have insulitis, infiltrating mononuclear cells could be responsible. Indeed, after culture of such islets the mononuclear cell infiltrate disappears and the islets show a restored glucose metabolism [16]. In order to further investigate the role of T lymphocytes in the mechanisms behind the islet-impaired metabolism, we treated female NOD mice with different monoclonal antibodies (MoAbs), i.e. MoAbs directed against the β-chain on the $\alpha\beta$-T cell receptor ($\alpha\beta$-TcR), which is used by a majority of peripheral T cells, or MoAb against CD4 and CD8 proteins, which are used by distinct T-cell subsets in the periphery. NOD mice injected with normal rat IgG or phosphate-buffered saline (PBS) served as controls, i.e. they represented the diabetes-prone, untreated female NOD mouse. Ten days

Table 1. Intravenous glucose tolerance test in female NOD mice treated with different monoclonal antibodies

Group	Time (min)			
	0	15	60	120
	Glucose (mmol/l)			
α-αβTcR	2.89 ± 0.16	23.4 ± 1.23	4.99 ± 0.85	3.60 ± 0.41
α-CD4	3.31 ± 0.29	22.5 ± 1.26	4.36 ± 0.41	4.11 ± 0.71
α-CD8	3.73 ± 1.06	22.1 ± 1.18	5.41 ± 0.49	5.30 ± 2.12
IgG	3.14 ± 0.26	22.3 ± 1.48	5.65 ± 0.67	3.69 ± 0.39
PBS	3.95 ± 0.92	23.0 ± 0.76	4.08 ± 0.36	5.58 ± 1.96

Female 12- to 13-week-old NOD mice were given glucose 2.5 g/kg body weight of a 30% glucose solution. Blood samples were obtained from the tail tip and measured using a blood glucose pen at time points 0, 15, 60 and 120 min. Values are given as means ± SEM of 8 to 10 animals. TcR, T-cell receptor; PBS, phosphate-buffered saline

Table 2. Pancreatic islet morphology in female NOD mice treated with different monoclonal antibodies

Group	Islet morphology rank (% of animals)				
	n	A	B	C	D
anti-αβTcR	9	78	22	0	0
anti-CD4	9	11	89	0	0
anti-CD8	7	14	57	29	0
IgG	8	0	12.5	75	12.5
PBS	11	9	18	73	0

The islet morphology of each animal was ranked according to four arbitrary classes: A, normal islet structure; B, mononuclear cell infiltration in the periinsular area; C, mononuclear cell infiltration in a majority of islets, i.e. insulitis; D, only a few residual islets exhibiting an altered islet architecture with cells often containing pyknotic nuclei or showing other signs of degeneration. TcR, T-cell receptor; PBS, phosphate-buffered saline. *n*, denotes number of animals

after the initial MoAb injections, islets were isolated and the Beta-cell function evaluated.

Materials and methods

Animals

Female NOD mice, aged 12- to 13-weeks, from a inbred local colony established in Uppsala in 1988, were used. Originally, three breeding pairs of inbred NOD mice were obtained from the Clea Company (Aobadi, Japan). The current cumulative diabetes incidence in the females is 35% at 28 weeks and 42% at 32 weeks. The mice in the study were randomized and litter-matched, i.e. one mouse from each litter received one of the five different treatments described below. Five to six animals were kept to each cage with free access to tap water and pelleted food (Type R3; Ewos Södertälje, Sweden). In some control experiments non-diabetes prone male NMRI mice (ALAB, Sollentuna, Sweden) were used.

MoAbs and treatment protocol

Three different antibodies were used: a hamster IgG MoAb directed against a monomorphic region of the β-chain of the T-cell receptor (hybridoma line H57-597) [17], a rat IgG anti-CD4 (GK 1.5) MoAb [18] and a rat IgG anti-CD8 (H35-17.2) MoAb [19]. Mice injected with normal rat IgG (Sigma Chemical Co., St. Louis, Mo., USA) and mice injected with phosphate-buffered saline (PBS) served as control groups. The MoAbs and normal rat IgG were dissolved in PBS and injected i.p. 9 and 5 days prior to killing. The amount of protein administered was 400–500 μg in 0.1 ml PBS per injection.

Intravenous glucose tolerance test and pancreas collection

Two days before the mice were killed a glucose tolerance test was performed. The mice were injected in the tail vein with 2.5 g/kg body weight glucose in a 30% (weight/volume, w/v) solution. Blood samples were obtained from the tail tip at 0, 15, 60 and 120 min and analysed using a blood glucose pen (ExacTech; Baxter Travenol Laboratories, Deerfield, Ill., USA). The mice were killed by cervical dislocation and the pancreata dissected out. A small piece of the pancreas was cut and quickly frozen for morphological studies of cryostate sections. The remaining pancreatic tissue (≈ 80%) was used for islet isolation.

Islet insulin release, insulin and DNA content

The pancreas was treated with collagenase (Boehringer Mannheim, Mannheim, FRG) and islets were isolated and hand-picked using a braking pipette [20]. The islets were immediately used for various experiments, except for the islets exposed to interleukin-1 β. For estimation of the insulin release capacity after glucose stimulation, islets were incubated in groups of 10 each in triplicate and placed in sealed glass vials containing 0.25 ml of Krebs Ringer bicarbonate buffer [21] supplemented with 10 mmol/l Hepes and 2 mg/ml bovine serum albumin (BSA; Miles, Slough, UK), hereafter referred to as KRBH. During the first hour of incubation at 37 °C (O_2:CO_2; 95:5) the KRBH medium contained 1.7 mmol/l glucose. The medium was then gently removed and replaced by 0.25 ml of KRBH supplemented with 16.7 mmol/l glucose and the incubation continued for a second hour. The insulin concentration of the medium was determined by RIA [22]. The inter-assay variation of the RIA was 7% and the intra-assay variation was 4%. In each experimental group the insulin secretion was calculated as the mean of the three incubation vials. After the insulin release experiments the islets were recovered and pooled in groups of 30 and disrupted by sonication in 0.2 ml redistilled water. An aliqout of the homogenate was mixed with acid-ethanol and the insulin extracted overnight at 4 °C. DNA was measured fluorophotometrically in another fraction of the water homogenate [23, 24].

Glucose oxidation rates

Three groups of 10 islets each were incubated in glass vials containing KRBH without BSA, but supplemented with D-[U-^{14}C]glucose (Amersham International, Amersham, UK) and non-radioactive glucose to a final concentration of either 1.7 or 16.7 mmol/l glucose. The vials were inserted into glass scintillation flasks, gassed (O_2:CO_2; 95:5) and sealed tightly. The flasks were incubated for 90 min at 37 °C until the oxidation was stopped by injection of 100 μl of 0.05 mmol/l antimycin A (Sigma) into the glass vials. $^{14}CO_2$ formed by the islet metabolism was released from the incubation medium by injection of 100 μl of 0.4 mol/l Na_2HPO_4 (pH 6.0) and trapped in 250 μl Hyamine 10-X (Packard Instruments, Downers Grove, Ill., USA) during a further 120-min incubation. Ten millilitres of Econofluor (New England Nuclear, Boston, Mass., USA) was added and the radioactivity measured by liquid scintillation counting.

For measurements of splenocyte glucose oxidation, splenocytes were washed out from the spleen through repeated flushing of medium RPMI 1640 (Flow Laboratories, Irvine, UK) with a syringe. Erythrocytes were lyzed by treating the cell suspension with ammonium chloride (0.19 mol/l) for 10 min at 4 °C. The remaining cells were washed with RPMI 1640 and resuspended in the same medium plus 10% (v/v) fetal calf serum (Flow). The cells were kept for 60 min at 37 °C in humified air in an atmosphere of 5% CO_2 in cell culture dishes that allow cell attachment (Nunc, Roskilde, Denmark). The cells were subsequently divided into two groups and the

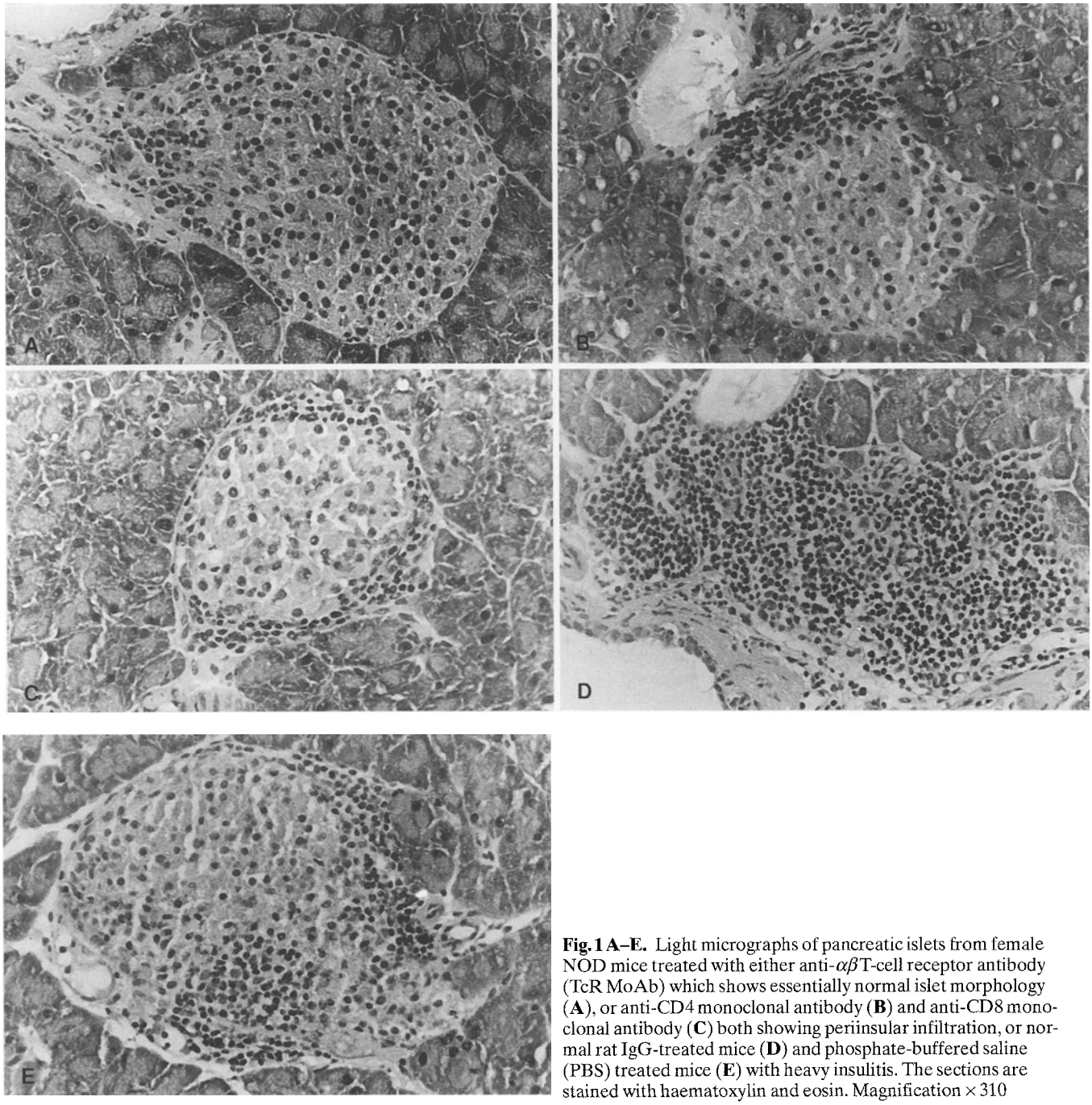

Fig. 1 A–E. Light micrographs of pancreatic islets from female NOD mice treated with either anti-$\alpha\beta$ T-cell receptor antibody (TcR MoAb) which shows essentially normal islet morphology (**A**), or anti-CD4 monoclonal antibody (**B**) and anti-CD8 monoclonal antibody (**C**) both showing periinsular infiltration, or normal rat IgG-treated mice (**D**) and phosphate-buffered saline (PBS) treated mice (**E**) with heavy insulitis. The sections are stained with haematoxylin and eosin. Magnification × 310

mitogen concanavalin A (5 μg/ml; Pharmacia Fine Chemicals, Uppsala, Sweden) was added to one group. The cells were then kept in culture tubes (Nunc) containing RPMI 1640 plus 10% fetal calf serum for 24 h at 37 °C in atmosphere of 5% CO_2 in humidified air. Cell viability was subsequently assessed by trypan blue exclusion and the cell number was counted in a Bürker chamber. Cells in groups of 2–5 × 10^6 were placed in each glass vial and the glucose oxidation rate was assessed as described above.

Glucose oxidation rates in islet infiltrating cells

In islets with a heavy insulitis, the infiltrating mononuclear cells form a translucent capsule around the endocrine component of the islets. When viewed under a stereomicroscope, this "capsule" can be clearly distinguished from the endocrine cells. In a separate series of experiments, islets were isolated from 13- to 18-week-old female NOD mice and infiltrating mononuclear cells composing this "capsule" were microdissected free from the remaining part of the islet. Approximately one-half of this material was used to measure glucose oxidation rates, and the other half was used for DNA and insulin content determinations in cellular homogenates.

Interleukin-1 β exposure

In these experiments pancreatic islets were isolated from untreated 13-week-old female NOD mice and male NMRI mice. The islets were kept free floating in culture medium RPMI 1640 containing 10% (v/v) fetal calf serum and antibiotics [25]. The culture medium

Table 3. DNA and insulin contents and glucose-stimulated insulin release of pancreatic islets isolated from female NOD mice treated with different monoclonal antibodies

Group	DNA content	Insulin content	Insulin release		Ratio
			1.7 mmol/l glucose	16.7 mmol/l glucose	16.7/1.7 mmol/l glucose
	(ng DNA/10 islets)	(ng insulin/10 islets)	(ng insulin · 10 islets^{-1} · 60 min^{-1})		
anti-$\alpha\beta$TcR	400 ± 40.0[c]	918 ± 122	3.7 ± 0.8	20.3 ± 5.7	5.4 ± 1.1
anti-CD4	510 ± 30.0[b]	1107 ± 185	4.9 ± 0.2	15.6 ± 3.7	3.3 ± 0.9
anti-CD8	570 ± 70.0[a]	1155 ± 197	5.5 ± 1.9	20.7 ± 5.1	6.0 ± 2.1
IgG	700 ± 70.0	989 ± 217	3.7 ± 0.7	16.8 ± 2.4	5.2 ± 0.7
PBS	850 ± 90.0	1164 ± 241	5.2 ± 1.1	17.3 ± 2.6	4.1 ± 0.8

The islets were isolated from female 13- to 14-week-old NOD mice treated in vivo with different monoclonal antibodies. Islet DNA content was measured fluorophotometrically in water homogenates of the islets and the insulin content was determined by RIA in acid ethanol extracts of the homogenized islets. The islet insulin release was measured by incubating islets in groups of 10 in medium containing 1.7 mmol/l glucose, after 60 min the medium was removed and the islets were incubated for another 60 min in medium containing 16.7 mmol/l. The insulin concentration was determined by RIA. Values are given as means ± SEM for 8–10 animals. [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ vs the phosphate-buffered saline (PBS) group, using Student's unpaired t-test. TcR, T-cell receptor

was changed every second day and after 7 days in culture, islets in groups of 80–120 were subsequently transferred to new culture dishes and maintained for a 48-h experimental period. Islets allocated to the experimental group were cultured as described above, but with the addition of 10 ng/ml human recombinant interleukin-1β (IL-1β) to the medium. IL-1β was kindly provided by Dr. K. Bendtzen (Laboratory of Medical Immunology, Copenhagen, Denmark). After 48 h, rates of islet glucose oxidation were measured. A biological assay [26] showed that 1 ng/ml of the cytokine had an activity of approximately 50 U/ml as compared with an interim international standard IL-1β preparation (NIBSC, London, UK).

Pancreatic islet morphology

At least 25 sections, 7 μm thick, of the cryofixed pancreas were cut and stained with haematoxylin and eosin. The pancreatic morphology was examined and ranked in accordance with a classification previously described in detail [27, 28], with the examiner unaware of the origin of the pancreatic sections. Class A denotes a normal islet morphology; class B denotes a mononuclear cell infiltration in the periinsular area; class C denotes a heavy mononuclear cell infiltration into a majority of the islets i. e. insulitis, and class D denotes only a few residual islets remaining, often displaying an altered islet architecture and containing pyknotic nuclei or showing other signs of cell degeneration.

Statistical analysis

Data are presented as means ± SEM and groups of data were compared using Student's unpaired t-test. When incubations were performed in duplicate or triplicate, a mean was calculated and considered as one separate observation.

Results

Intravenous glucose tolerance test

Blood glucose concentrations were not different among the groups before glucose injection (Table 1). All groups of mice showed similarly elevated blood glucose concentrations 15 min after the glucose load and after 60 and 120 min the glucose levels had returned to their initial levels.

Pancreatic islet morphology

The pancreatic islet morphology of mice treated with either normal rat IgG or PBS showed a heavy infiltration of mononuclear cells, e.g. insulitis in most animals (Table 2; Fig. 1). Treatment with the MoAbs markedly reduced the degree of infiltrating cells. The anti-$\alpha\beta$ TcR MoAb treatment led to a complete normalization of the islet morphology in a majority of the mice (Table 2; Fig. 1), whilst 80–90% of the mice treated with anti-CD4 and anti-CD8 MoAbs exhibited some degree of mononuclear cell infiltrate especially in the periinsular area (Table 2; Fig. 1).

Islet insulin and DNA content and islet insulin release

All three MoAbs markedly reduced the DNA content of the isolated islets (Table 3). Since there was no parallel reduction in islet insulin content (Table 3), this decrease in islet DNA probably corresponds to a depletion of infiltrating mononuclear cells, as previously suggested for cultured NOD islets [16, 29]. For the anti-$\alpha\beta$ TcR MoAb treated mice the reduction was around 400 ng DNA/10 islets. Assuming a DNA content of 6 pg per cell [30], 400 ng of DNA would correspond to approximately 60,000 infiltrating mononuclear cells per 10 islets.

There was no significant difference in the insulin release in vitro between the various groups (Table 3). Thus, the basal insulin secretion at 1.7 mmol/l glucose was similar and all groups of islets responded with a four- to five-fold elevated insulin release following stimulation with 16.7 mmol/l glucose. In order to evaluate insulin release capacity in mice older than 14 weeks, a separate series of experiments with non-treated 16-week-old female NOD mice was performed. The insulin release at 1.7 mmol/l and 16.7 mmol/l glucose were 4.9 ± 0.8 and 11.4 ± 0.8 ng insulin · 10 islets^{-1} · 60 min^{-1} ($n = 8$) respectively, corresponding to a two-fold increase in insulin response. The insulin content was 899 ± 143 ng insulin/10 islets and the DNA content was 837 ± 157 ng DNA/10 islets.

Table 4. Rates of glucose oxidation in pancreatic islets isolated from female 13- to 14-week-old NOD mice treated with different monoclonal antibodies

Group	1.7 mmol/l glucose	16.7 mmol/l glucose	Ratio (16.7/1.7 mmol/l glucose)
	(pmol glucose · 10 islets^{-1} · 90 min^{-1})		
anti-$\alpha\beta$TcR	41 ± 5.0[b]	367 ± 54	9.4 ± 0.9[b]
anti-CD4	52 ± 8.0[b]	335 ± 60	7.8 ± 1.8[b]
anti-CD8	72 ± 21[b]	335 ± 52	7.0 ± 2.1[a]
IgG	180 ± 33	283 ± 39	2.2 ± 0.5
PBS	195 ± 12	322 ± 38	1.7 ± 0.2

The islets were incubated in KRBH at either 1.7 or 16.7 mmol/l glucose in the presence of D-[U-^{14}C]glucose for 90 min at 37°C (O_2/CO_2; 95:5). Values are means ± SEM for 7 to 9 animals. [a] $p < 0.01$ and [b] $p < 0.001$, vs the phosphate-buffered saline (PBS) group using Student's unpaired t-test. TcR, T-cell receptor

Table 5. Rates of glucose oxidation in splenocytes isolated from untreated female NOD mice

Con A	1.7 mmol/glucose	16.7 mmol/l glucose	Ratio (16.7/1.7 mmol/l glucose)
	(pmol glucose · 60,000 cells^{-1} · 90 min^{-1})		
−	8.8 ± 1.0	17.8 ± 1.8	1.87 ± 0.14
+	54 ± 8.0[a]	108 ± 8.6[a]	2.14 ± 0.18

The splenocytes were isolated from 12- to 13-week-old female NOD mice. The cells were kept in culture for 24 h with or without concanavalin A (Con A; 2.5 μg/ml) present, before the glucose oxidation rate was determined. 2–5 × 10^6 splenocytes were incubated in KRBH at either 1.7 or 16.7 mmol/l glucose in the presence of D-[U-^{14}C]glucose for 90 min at 37°C (O_2:CO_2; 95:5). Values are means ± SEM for eight experiments. [a] $p < 0.001$ vs non-Con A treated splenocytes using Student's unpaired t-test

Islet glucose oxidation rate

The basal glucose oxidation rates at 1.7 mmol/l glucose in the groups of mice treated with MoAbs was reduced by approximately 65–75% compared to the PBS-treated mice (Table 4). However, at 16.7 mmol/l glucose the glucose oxidation rates were similar in all groups. When a ratio between high and low glucose oxidation rates was calculated, the groups treated with MoAbs showed significantly higher values than the control groups.

Splenocyte glucose oxidation rate

In order to assess the possible contribution of glucose oxidized by infiltrating mononuclear cells to the total islet glucose oxidation observed in the control infiltrated islets (Table 4), the glucose oxidation rates of splenocytes from 12- to 13-week-old non-treated mice were also examined. The values are expressed to correspond to the estimated difference in cell content (60,000 cells/10 islets) between the PBS and anti-$\alpha\beta$TcR MoAb treated mice. The glucose oxidation rates in non-activated splenocytes was low both at 1.7 and 16.7 mmol/l glucose (Table 5). In splenocytes stimulated with concanavalin A the glucose oxidation rates were elevated approximately six times at both glucose concentrations. The ratio high/low glucose was nevertheless unaffected by the presence or absence of concanavalin A.

Glucose oxidation rates in islet infiltrating cells

Since the islet infiltrating mononuclear cells may be different from the mononuclear cells present in the spleen, it is unclear whether the splenocyte glucose oxidation rates described above correspond to the actual glucose oxidation rates of the NOD islet mononuclear cells. In order to address this issue, areas of infiltrating mononuclear cells were microdissected from heavily infiltrated pancreatic islets. This material was obtained both from 13-week-old and 18-week-old female NOD mice (a total of four experiments were performed; each experiment comprised islet infiltrates pooled from pancreatic glands of two to four NOD mice). Since the results obtained were similar, irrespective of the age of the animals, the data are pooled. Beta cells were also present in the microdissected material, as assessed by the ratio between insulin and DNA contents. Thus, the insulin per DNA content (ng/ng) of the infiltrates was around 8% of the values observed in whole pancreatic islets isolated in parallel from the same animals (data not shown). The glucose oxidation rates of the islet infiltrates was 47 ± 21 pmol glucose · μg DNA^{-1} · 90 min^{-1} at 1.7 mmol/l glucose and 68 ± 13 pmol glucose · μg DNA^{-1} · 90 min^{-1} at 16.7 mmol/l glucose. Based on these data, and assuming that islets isolated from control NOD mice contain approximately 60,000 mononuclear cells per 10 islets, it can be estimated that the infiltrating cells might oxidize about 20 pmol glucose · 90 min^{-1} at 1.7 mmol/l glucose, and about 30 pmol glucose · 90 min^{-1} at 16.7 mmol/l glucose.

Islet glucose oxidation rate after exposure to IL-1 β

Islets isolated from non-treated NOD mice were pre-cultured for 6 days in order to deplete the mononuclear cell infiltrate [16]. This culture period induced a clear reduction in the basal glucose oxidation rate as compared with the PBS and IgG group in Table 4. Islets exposed to IL-1 β for 48 h had a glucose oxidation rate at 1.7 mmol/l glucose three times higher that seen in non-treated islets, whereas at high glucose there was no difference in the oxidative rates (Table 6). As a consequence the high/low glucose ratio was decreased after cytokine exposure. Glucose oxidation experiments performed in parallel with islets from non-diabetes prone NMRI mice, used as an external control, showed a similar response to IL-1 β as that obtained with the NOD mouse islets.

Discussion

The aim of this study was to investigate the role of infiltrating T cells in impaired Beta-cell function in islets isolated from NOD mice. We chose to study the action of three different MoAbs directed against T-cell populations in this context. The $\alpha\beta$-T cell MoAb has been shown to abolish insulitis and prevent diabetes in NOD mice [31].

Table 6. Rates of glucose oxidation in pancreatic islets isolated from female NOD or male NMRI mice cultured with or without 10 ng/ml interleukin-1 β (IL-1 β)

Strain	IL-1 β	1.7 mmol/l glucose (pmol glucose · 10 islets^{-1} · 90 min^{-1})	16.7 mmol/l glucose (pmol glucose · 10 islets^{-1} · 90 min^{-1})	Ratio (16.7/1.7 mmol/l glucose)
NOD	–	55 ± 7.0	413 ± 30	10.1 ± 1.7
	+	153 ± 25[a]	496 ± 35	4.2 ± 0.3[a]
NMRI	–	36 ± 7.0	448 ± 37	14.3 ± 3.1
	+	110 ± 14[b]	537 ± 58	5.2 ± 0.8[b]

The islets were isolated from female 13- to 14-week-old NOD and 12-week-old male NMRI mice. The islet glucose oxidation was determined in cultured control islets and in islets cultured for 48 h in the presence of 10 ng/ml IL-1 β. The islets were incubated in KRBH at either 1.7 or 16.7 mmol/l glucose in the presence of D-[U-^{14}C]glucose for 90 min at 37°C (O_2:CO_2; 95:5). Values are means ± SEM for five animals. [a] $p < 0.05$, [b] $p < 0.001$ vs non-cytokine treated control islets using Student's unpaired t-test

The anti-CD4 MoAb depletes circulating CD4$^+$ T cells by 90% and prevents diabetes in NOD mice [32]. The anti-CD8 MoAb has previously been shown to prevent the induction of diabetes by cyclophosphamide [33] or the transfer of diabetes by spleen cells from diabetic animals in NOD mice [34]. NOD mice injected with rat IgG or PBS served as controls in order to follow the natural history of diabetes-prone female NOD mice.

The present data show that there were no differences in the glucose-stimulated insulin release among the experimental and control groups. It is noteworthy that the islets isolated from control NOD mice had a higher insulin release than previously observed in female NOD mice of the same age [16]. This discordance may be explained by the delayed onset of clinical diabetes in our present colony. The altered insulin release from 16-week-old female NOD mouse islets actually indicates a later deterioration in insulin secretion. An early islet dysfunction in the form of disturbed glucose oxidation rate was nevertheless observed in 13- to 14-week-old NOD mice along with insulitis. Islets isolated from control NOD mice presented an abnormally high basal glucose oxidation rate when compared to all three MoAb-treated groups. As a consequence a low ratio between high and low glucose oxidation rates was observed in the control groups. Treatment with all three anti-T cell MoAbs reduced the islet mononuclear cell infiltrate and restored the islet glucose metabolism. The reduction in islet cell infiltration was probably due to a depletion of pre-existing mononuclear cells, since all untreated 12- to 13-week-old NOD mice displayed periinsular infiltration or insulitis. It is a reasonable assumption that infiltrating lymphocytes are thus responsible for the observed effects in the glucose oxidation rates. Interestingly, the anti-$\alpha\beta$ TcR MoAb, which recognizes the target T-cell population in the periphery, showed the greatest effect on both insulitis and the islet glucose oxidation rates. However both anti-CD8 and anti-CD4 MoAbs also showed significant effects. This may indicate that both T-cell subsets participate in the functional effects reported above. This action of T cells could be due either to a direct contribution to the total islet oxidation, or to a mononuclear cell-induced alteration in Beta-cell function. In order to test the first possibility, we initially studied the glucose metabolism in concanavalin A stimulated splenocytes. Despite the fact that the mitogen caused an increased glucose oxidation rate in these cells, a calculated lymphocyte contribution only partly explained the increased basal islet glucose oxidation rate. Similar results were obtained with mononuclear cells microdissected from heavily infiltrated islets. Thus, a calculated contribution by these cells to the total islet glucose oxidation rate accounts for only about 10% of the values observed in whole islets isolated from 12- to 13-week-old female NOD mice. Finally, it cannot be excluded that a small amount of leaking insulin stimulates glucose uptake and basal oxidation rates in islet infiltrating cells. Indeed there are reports of activated lymphocytes presenting insulin receptors [35]. However, the present findings of similar insulin contents and basal insulin release rates of islets isolated from the different experimental groups, argues against an extensive leakage of insulin.

Both a direct cell-to-cell interaction and an indirect interaction by, for instance, secreted cytokines may mediate the islet functional defect observed in NOD infiltrated islets. Cytokines are expected to be present in the vicinity of Beta cells during the insulitis process and are likely to mediate CD4$^+$ T-cell related effects. Indeed, recent data suggest that there is increased transcription of mRNA for tumour necrosis factor-α and IL-1 in islets of pre-diabetic BB rats and NOD mice [36, 37]; (Sandler S, Bendtzen K, Forsbeck E, Mares J, Strandell E, Welsh N, Welsh M, unpublished observation). A previous report showed that in vitro exposure of NOD islets to 50 or 100 U/ml of IL-1 β, decreased insulin release by 40%, compared to a non-treated control group [38]. Based on these results and other reports on the effects of IL-1 on Beta cells [39, 40], we tested the in vitro effect of IL-1 β on NOD mouse islet oxidation. Cultured islets exposed to IL-1 β showed an altered glucose oxidation rate which was comparable to that seen in islets isolated from NOD mice treated with rat IgG or PBS, i.e. a high basal glucose oxidation and a failure to further increase the glucose metabolism in response to high glucose. These findings suggest that exposure of non-infiltrated islets to one of the potential immune mediators of islet inflammation in early Type 1 diabetes, can induce a pattern of altered glucose metabolism similar to that observed in islets isolated from NOD mice during the "pre-diabetic" period. IL-1 β is mainly secreted by macrophages and it is believed that activated T cells produce cytokines that stimulate monocytes and macrophages to make IL-1 and TNF [41].

Altogether, these and the finding of low glucose metabolism observed in mononuclear cells, suggest that the in-

crease in basal glucose oxidation in the NOD mouse occurs primarily at the islet endocrine cell level. Thus, alterations in the islet glucose oxidation rate may be an early sign in NOD mouse islets, eventually preceding the deterioration of insulin secretion and cell death. These early alterations in glucose metabolism can be reversed in vivo by MoAbs directed against effector lymphocytes. This suggests that reversible changes in pancreatic Beta-cell function induced by infiltrating mononuclear cells may precede the induction of both glucose intolerance and Beta-cell destruction in NOD mice. The cellular events underlying the observed increase in basal glucose metabolism remain unclear. In the case of pancreatic islets exposed in vitro to rIL-1 β, it has been shown that the cytokine increases islet gene expression and protein synthesis for heat-shock proteins (38, 40, 42, 43), the c-fos protooncogene [44], superoxide dismutase [45] and reg protein [46]. Perhaps all these energy requiring events are accompanied by an enhanced glucose metabolism. Extrapolating these findings to Beta cells exposed in vivo to an autoimmune assault, it can be envisaged that the observed increase in basal glucose oxidation in NOD mouse islets may be due to an increased demand on substrate metabolism, for synthesis of different defense proteins required by the Beta cells during this period, which eventually precedes overt diabetes.

The present data should also be considered in relation to recent attempts to block the anti-islet autoimmune reaction in Type 1 diabetic patients [47]. The induction of remission of the insulin-dependent state has been interpreted as reflecting either improvement of peripheral insulin resistance or an islet Beta-cell regeneration for which there is too little, if any, direct evidence. Our study in the NOD mouse shows that part of the metabolic consequences of the anti-islet Beta-cell autoimmune reaction reflect functional defects of Beta cells not yet destroyed by the process of insulitis. These functional defects can be reversed by eliminating infiltrating lymphocytes and may contribute to the metabolic remission reported in patients with early Type 1 diabetes.

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