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Transfer of Experimental Autoimmune Insulitis by Spleen Cells in Mice

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Summary. We have tested whether experimental insulitis induced by multiple subdiabetogenic injections of streptozotocin can be transferred by lymphocytes to normal recipients. C57BL/6J mice were treated on 5 consecutive days with 40 mg streptozotocin /kg body weight. 5×10^7 nucleated spleen cells from 20 animals which had developed hyperglycaemia with concomitant insulitis three weeks after the first streptozotocin-injection, were transferred into congenic thymusless C57BL/6J-*nu/nu* mice. The cell transfer led to lymphocytic infiltrations of pancreatic islets in 75% of the recipients. Hyperglycaemia was not observed. It is concluded that low-dose streptozotocin treatment induces cellular immune reactions against pancreatic islets.

Key words: Insulitis, lymphocyte transfer, cellular immunity, streptozotocin, experimental diabetes mellitus, glucose intolerance.

Repeated injections of low doses of streptozotocin induce hyperglycaemia with concomitant insulitis in some mouse strains [1, 2]. Several similarities have been reported to exist between this type of experimental insulitis and diabetes in mice and the insulin-dependent diabetic syndrome in humans [3].

The manifestation of low-dose streptozotocin-induced diabetes could not be prevented by blocking the B-cell cytotoxic activity of streptozotocin with 3-o-methyl-D-glucose. However, the administration of antilymphocyte serum completely protected animals from the development of diabetes [4]. Thus, cellular immune reactions against pancreatic islets appear to be involved in the aetiology of low-dose streptozotocin-induced diabetes. It has been suggested that the immune reactions are directed against altered structures of islet cells which would be generated during streptozotocin-treatment [1, 4].

The purpose of our study was to investigate whether immune reactions involved in the low-dose streptozotocin-induced diabetes are transferable by spleen cells of streptozotocin-treated mice to normal athymic recipients. If this is the case it would suggest that autoimmune lymphocytes with specificity for pancreatic islets are involved in streptozotocin insulitis.

Materials and Methods

Mice

Male C57BL/6J/Bom[f] and C57BL/6J/Bom-*nu/nu* of 8-12 weeks of age were received from Gl. Bomholtgard Ltd., Ry, Denmark. Mice were kept under conventional conditions with water and food ad libitum.

Streptozotocin Treatment

Donor mice received five doses of 40 mg streptozotocin (Boehringer, Mannheim, Germany) /kg body weight on consecutive days. Intraperitoneal injections were given between 0900 and 1100 h to nonfasted mice, immediately after dissolving the chemical in sodium citrate buffer (25 mmol/l at pH 4.0). Control groups received injections of the buffer alone or were untreated.

Transfer Procedure

Three weeks after the first streptozotocin-injection all animals had developed diabetes as judged from blood glucose estimation. They were sacrificed and their spleens removed. Spleen cells were isolated by shredding the spleen with steel wire mesh (gauge 0.12 mm) followed by two washes of cells at 200 g for 10 min in Hank's buffer. Viability of cells was between 85 and 90% as judged from eosin dye exclusion tests [5].

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Table 1. Summary of blood sugar values and morphological characteristics in donors and recipients

Donor mice						Recipient mice					
Strain	n	Streptozotocin treatment	Hyperglycaemia	Insulitis	Periductulitis	Strain	n	Glucose intolerance	Hyperglycaemia	Insulitis	Periductulitis
Test C57BL/6J	20	+	20	20	2	C57BL/6J *nu/nu*	20	5	0	10	7
Control C57BL/6J	10	–	0	0	0	C57BL/6J *nu/nu*	10	2	0	1	0

Spleen cells, 5×10^7, of a single donor mouse were transferred into congenic recipients. Donor mice were sacrificed 3 weeks after the first streptozotocin-injection. Analysis of recipient mice was 2 weeks after the cell transfer. In the control experiment donor mice were sham-treated with injections of citrate buffer. n = numbers per group

For cell transfer spleen cells from individual donor mice were kept separately. Each recipient mouse received IP an aliquot of the spleen cell isolate containing 5×10^7 live nucleated cells in a total volume of 0.5 ml. Recipients were sacrificed for histological examination two weeks after cell transfer. Only recipients of spleen cells from donors with insulitis were studied.

Blood Glucose Estimation

Blood of non-fasted donor mice was sampled between 0900 and 1100 h one day before the first streptozotocin-injection and two days before the transfer of spleen cells. Glucose tolerance tests on non-fasted animals were performed between 0900 and 1100 h by injecting 0.014 mol of glucose/kg body weight IP. Blood was drawn at 0, 30 and 60 min one day before the transfer and 14 days later. Glucose was determined in an Autoananalyser (Technicon Instruments Corp., New York, USA), using the GOD-perid method of Boehringer, Mannheim (Germany). Animals with basal blood glucose values under 11 mmol/l were considered normal, over 11 mmol/l hyperglycaemic. The border line for glucose intolerance of C57Bl/6J-*nu/nu* mice was set for a 60 min value higher than 14 mmol/l; the average 60 min value of 26 untreated nude mice was 9.0 ± 0.5 mmol/l.

Histology

For histological examination the pancreata of donors and recipients were dissected, carefully attached to a piece of cork, immediately fixed in Bouin solution and embedded in paraffin. Pancreatic sections always included both head and tail region. They were stained with haemotoxylin-eosin and Giemsa solution [for routine observation (5)], paraldehyde-fuchsin [to differentiate between A- and B-cells (6)] and van Gieson stain [to detect fibrosis (5)].

Eight to eighteen islets were observed per section. All were screened for pathological changes. Insulitis was arbitrarily classified either as weak (10–20 lymphocytes observed per islet section) or as strong (more than 20 lymphocytes per islet section). Pancreatic sections were read by two investigators without knowing the experimental protocol.

In addition, pieces of the livers and kidneys of recipient mice were fixed in formalin and embedded in paraffin. Staining was performed with haematoxylin-eosin.

Results

C57BL/6J mice that had developed hyperglycaemia three weeks after the first of 5 injections of 40 mg streptozotocin/kg body weight were sacrificed and their spleen cells transferred into congenic C57BL/6J-*nu/nu* mice. Table 1 summarises the results of 5 separate transfer experiments. It is apparent that most recipients of lymphocytes from donors with streptozotocin-induced diabetes and insulitis developed insulitis and/or periductulitis whereas almost all recipients of control lymphocytes showed no pathological changes in the islets. The same result was obtained in non-treated C57BL/6J-*nu/nu* mice (cf. Fig. 2).

A detailed analysis of donor and recipient histology is given in Table 2. In recipient mice with insulitis between 20 and 80% of islets (average 45%) were found to be infiltrated by lymphocytes.

Insulitis in recipient mice had similar characteristics to that in donor mice. In both cases infiltrating cells were mostly lymphocytes as judged by light microscopy. Small lymphocytes were characterised by small cytoplasmic content of the cells, which allowed differentiation from cells being rich in cytoplasm, (such as islet cells, plasma cells, granulocytes and histiocytes).

In Figure 1 the various types of inflammatory responses observed in recipient mice are demonstrated. Lymphocytic infiltration was found within the islets (Fig. 1A), at the periphery of islets (Fig. 1B) and/or in areas between islet and ducts (Fig. 1B). Islets often showed signs of progressive degeneration.

Histological examination of the livers revealed in both experimental and control groups slight activa-

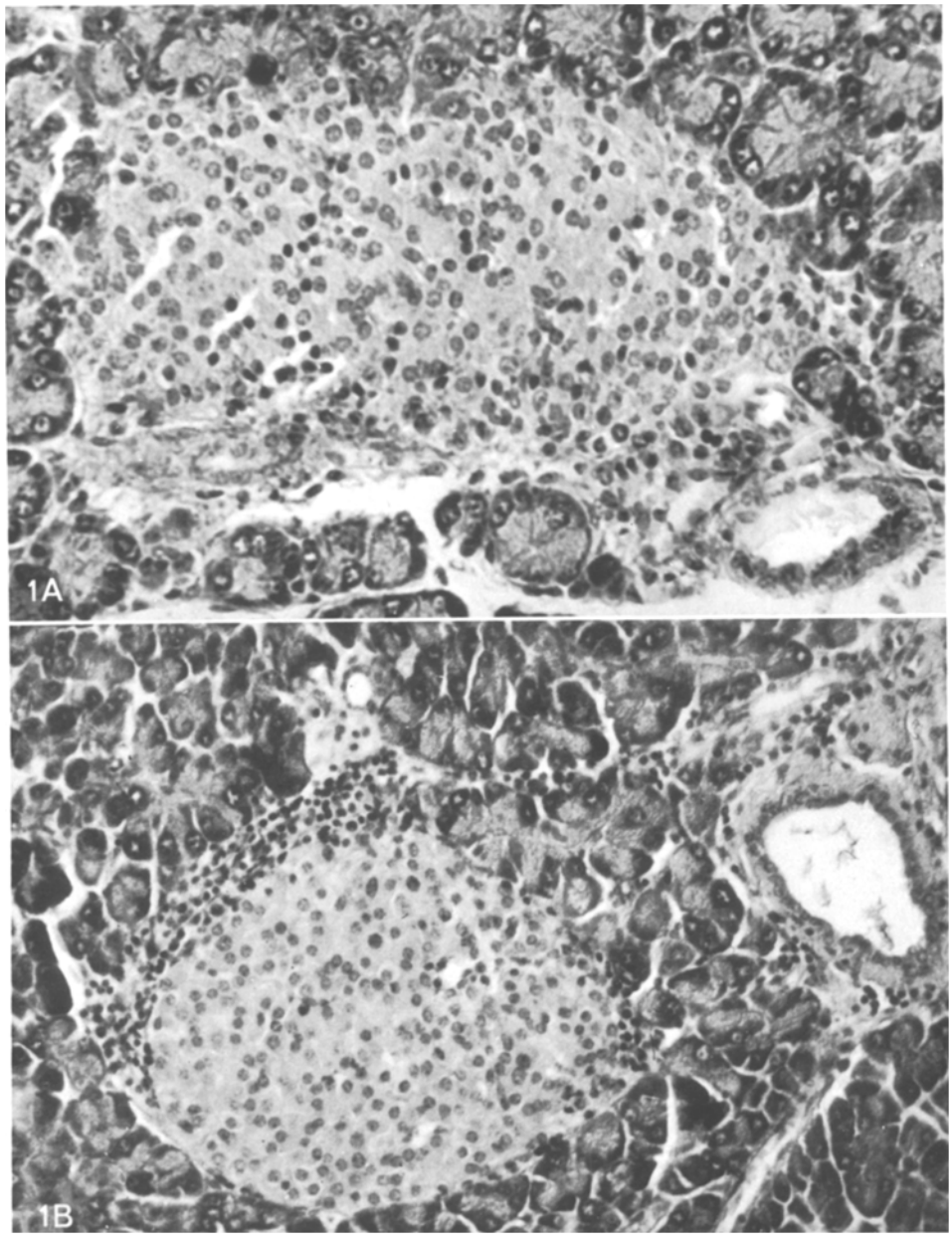

Fig. 1 A and B. Morphology of pancreatic inflammation in C57BL/6J-*nu/nu* recipient mice 14 days after transfer of 5×10^7 lymphocytes from streptozotocin-treated C57BL/6J donors. **A** Severe infiltration of lymphocytes, characterised by diffuse distribution of lymphocytes throughout the islet (magnification 300 ×, haematoxylin-eosin stain). **B** Infiltration at the periphery of the islet and around the duct (magnification 200 ×, haematoxylin-eosin stain)

tion of the Kupffer cells, but no signs of necrosis or hepatitis. The kidneys showed no abnormal changes.

In each recipient mouse glucose tolerance tests were performed before and after transfer of lymphocytes (Fig. 2). Glucose tolerance was normal at the onset of the experiments. A few recipients of spleen cells from diabetic donors developed abnormal glucose tolerance, but differences were not significant. None of the animals became hyperglycaemic during the observation period after cell transfer. No correlation between the degree of insulitis and the degree of glucose intolerance was apparent (Fig. 2).

Discussion

Lymphocytic infiltration in the islets of Langerhans were described as "insulitis" in human diabetics who died shortly after the onset of the disease [7, 8]. Insulitis may reflect an immune-mediated reaction.

Table 2. Individual analysis of islet morphology

Donor mice (Streptozotocin-treated)			Recipient mice		
No.	Periductulitis	Insulitis	Periductulitis	Insulitis	Number of islets with insulitis of total per section
1	−	+	+	−	0/11
2	+	+	+	−	0/14
3	−	(+)	+	−	0/12
4	−	(+)	+	(+)	4/ 8
5	−	(+)	−	(+)	2/ 9
6	−	+	−	−	0/ 9
7	−	(+)	−	+	3/14
8	+	+	+	−	0/15
9	−	+	+	+	3/ 8
10	−	(+)	−	−	0/13
11	−	(+)	(+)	−	0/18
12	−	+	−	−	0/10
13	−	+	−	+	18/18
14	−	+	−	+	8/12
15	−	+	−	(+)	3/13
16	−	+	−	+	8/12
17	−	+	+	+	4/12
18	−	+	−	+	3/13
19	−	+	−	−	0/12
20	−	+	−	−	0/12

Insulitis was arbitrarily classified as weak [(+)] with 10–20 lymphocytes found per islet section or strong [+] with more than 20 lymphocytes
For details see Table 1

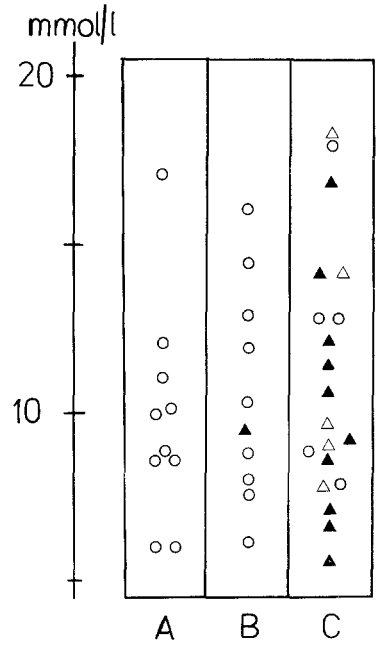

Fig. 2. Degree of insulitis or periductulitis and glucose intolerance. The blood glucose value 60 min after the glucose load is shown for individual C57BL/6J-*nu/nu* mice. Group A: untreated controls. Group B: control recipients 14 days after transfer of 5×10^7 lymphocytes of sham-treated C57BL/6J donors. Group C: recipients 14 days after transfer of 5×10^7 lymphocytes of low-dose streptozotocin-treated donors. ○ = no insulitis, △ = weak insulitis, ▲ = severe insulitis

Evidence for this is provided by our demonstration in mice that this phenomenon can be produced in healthy recipients following transfer of spleen cells from an affected animal.

Our finding that low-dose streptozotocin-induced insulitis can be transferred by lymphocytes to normal recipients argues in favour of an immune mediated mechanism being present. As lymphocytic infiltration was not found in the kidneys and livers of the recipients but only in their islets of Langerhans we conclude that specific cellular immune reactions against islet cells are involved.

Streptozotocin apparently induces in donor mice cellular immune reactions against parts of pancreatic islets which are present in both donor and recipient mice. If the insulitis in donor animals were due to lymphocytes reacting with new or altered determinants on islet cells – generated by the action of streptozotocin – transfer of the disease could not be achieved since normal recipients would lack the proper target determinant, unless there is sufficient cross-reaction between original and altered determinants.

Changes in antigenic structure of islet cells by streptozotocin nevertheless may be important for the induction of the autoimmune process. Thus it is known that immunisation of mice with foreign or altered islet tissue leads to cellular and humoral autoimmune reactions against their own islets ([9]; Kolb, Kiesel and Freytag, unpublished experiments).

It is of interest that histological characteristics of insulitis in low-dose streptozotocin-induced diabetic mice and in recipients of lymphocytes from such mice were similar. Lymphocytic infiltration was often observed at the periphery and/or at the ductular pole of islets. In 40% of the recipients periductular infiltration also occurred. This finding supports our suggestion that lymphocytes react with similar pancreatic structures in both donor and recipient animals. An ultrastructural analysis of the pancreas morphology in recipients is in progress.

In the present experiments a viral origin of insulitis observed in recipients cannot be excluded. Streptozotocin has been shown to activate C-type viruses [10]. Therefore, a transfer of lymphocytes may include the transfer of activated endogenous C-type virus.

A successful transfer of insulitis by lymphocytes has not been described before. However, a successful transfer of hyperglycaemia from low-dose strep-

tozotocin-treated mice to normal syngeneic and congenic recipients by means of lymphocytes has been reported [11, 12]. In the experiments presented here hyperglycaemia was not observed.

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