

## Originals

# Prevention and suppression of autoimmune pancreatic Beta-cell destruction in BB rats by syngeneic lymphocytes obtained from long-term normoglycaemic donors

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**Summary.** To prove whether a cell-mediated mechanism is responsible for maintaining long-term normoglycaemia in BB/OK rats with a proved immune attack (insulinitis, reduced Beta-cell volume), we transferred lymphocytes obtained from those rats into normoglycaemic diabetes-prone BB/OK rats or into diabetic BB/OK rats receiving a simultaneous syngeneic islet graft. Our results show the presence of a lymphocyte population in the long-term normoglycaemic BB/OK rats, which is able to arrest pancreatic Beta-cell destruction in diabetes-prone BB/OK rats detected by a decreased diabetes incidence following single lymphocyte transfusion. Syngeneic islets were destroyed by recurrence of the autoimmune process when transplanted into diabetic BB/OK rats. Lymphocytes obtained from long-term nor-

moglycaemic BB/OK rats were able to protect the syngeneic BB/OK islet graft from autoimmune destruction in diabetic BB/OK rats, whereas allogeneic islet destruction was not prevented. The phenotype of the effective lymphocyte population is not yet clear, but it is negative for RT6. We conclude that the mechanism responsible for maintaining normoglycaemia in long-term normoglycaemic BB/OK rats is cell mediated, because this property can be transferred to prevent autoimmune destruction of pancreatic Beta cells.

**Key words:** Islet transplantation, BB rat, autoimmune pancreatic Beta-cell destruction, lymphocyte transfer.

In an identical environment, only 30 to 60% of BB rats develop an insulin-dependent diabetes which is characterized by severe inflammation of pancreatic islets and a marked destruction of pancreatic Beta cells [1]. Despite an identical genetic background a portion of the animals did not develop hyperglycaemia, even when the rats were followed-up for more than 200 days. Recently, we observed insulinitis in all the BB/OK rats investigated independently, whether they developed hyperglycaemia or not [2]. This means that inflammation of islets does not necessarily lead to overt diabetes, suggesting that the BB/OK rats which did not develop diabetes (later referred to as long-term normoglycaemic BB rats) had arrested the immune destruction of pancreatic Beta cells spontaneously. Assuming that cell-mediated mechanisms were involved in such cessation processes, we isolated lymphocytes from these BB rats and transfused them into diabetes-prone BB rats or into diabetic BB rats receiving a simultaneous syngeneic islet graft, which is normally destroyed by recurrence of the autoimmune process [3–6].

## Materials and methods

### Animals

We divided 40 normoglycaemic diabetes-prone BB/OK rats (plasma glucose:  $6.9 \pm 0.2$  mmol/l) at an age of  $92 \pm 1$  days into two groups and included them in the lymphocyte transfusion study. Spontaneously diabetic BB/OK rats ( $n = 44$ , 24 male, 20 female) with a diabetes duration of  $>3$  weeks (repeated plasma glucose  $>20$  mmol/l, pancreatic insulin content  $<1.0$  pmol/mg wet/weight) served as recipients for the islet grafts. They were treated by a once daily injection of a long-acting insulin (heat-treated Berl-Insulin, VEB Berlin Chemie, Berlin, Germany) to prevent loss of body weight. Neonatal (8–12 days of age) BB/OK rats and neonatal LEW.1A rats were used for preparation of donor islets. Normoglycaemic BB/OK rats (plasma glucose:  $6.4 \pm 0.1$  mmol/l) with an age of  $232 \pm 2$  days were used as lymphocyte donors ( $n = 98$ , 55 male, 43 female). All animals were kept under semi-barrier conditions [7] and had free access to food (sterilized rat pellet chow R13, VEB Futtermittelwerke Altglienicke, FRG and vitamins) and sterilized water.

### Lymphocyte preparation

Lymphocytes were prepared from cervical lymph nodes, the spleen or peripheral blood as described earlier [8].

The number of cells for lymphocyte transfer was adjusted to  $1.6\text{--}2.5 \times 10^7$  per 2 ml RPMI 1640 containing 1% neonatal calf serum. Viability was > 98% as ascertained by trypan blue exclusion.

### Islet isolation and graft characterization

Donor pancreatic islets were prepared by fractionated collagenase digestion. The isolated islets were separated by centrifugation on a Dextran gradient [9] and hand-picked. For graft characterization islet insulin content [10] was determined.

### Lymphocyte transfusion into normoglycaemic diabetes-prone BB/OK rats

A mixture of splenocytes and cervical lymph node lymphocytes ( $2.47 \pm 0.44 \times 10^7$ ) was transfused into the inferior vena cava of 19 diabetes-prone BB rats (9 male, 10 female). Sham-transferred BB rats ( $n = 21$ , 11 male, 10 female) served as controls (infusion of 2 ml RPMI 1640). Plasma glucose (Glucose Analyzer, Beckman Instruments, Fullerton, Calif., USA), was monitored once a week up to more than 200 days (meaning 120 days after transfusion). Overt diabetes was diagnosed by a plasma glucose above 10 mmol/l on two consecutive days.

### Lymphocyte transfusion and islet transplantation into diabetic BB/OK rats

Two thousand hand-picked islets were grafted under the kidney capsules of diabetic BB/OK recipients. These were either untreated (control, syngeneic and allogeneic islet graft), transfused with a mixture of splenocytes and cervical lymph node lymphocytes (syngeneic and allogeneic islet graft;  $2.45 \pm 0.22 \times 10^7$  cells and  $2.01 \pm 0.05 \times 10^7$  cells) or peripheral blood lymphocytes (syngeneic islet graft;  $1.66 \pm 0.08 \times 10^7$ ). At the time of transplantation the recipients were surgically biopsied to obtain pancreatic tissue which was used to measure the pancreatic insulin content [11]. Plasma glucose was monitored three times a week up to day 21 and then once a week up to day 120. Islet destruction was considered as being the first of two consecutive days when plasma glucose exceeded 10.0 mmol/l. Hyperglycaemic animals were killed, and the pancreas was taken for insulin extraction [11]. In normoglycaemic animals, the grafts were removed after 120 days for insulin extraction [11]. Plasma glucose of the nephrectomized rats was followed-up for further three consecutive days.

### Statistical analysis

The results are given in mean values  $\pm$  SEM and a Student's *t*-test was used to check statistical significance.

## Results

As Table 1 shows, the diabetes incidence of sham-transferred animals was 33%. Lymphocyte transfusion decreased diabetes manifestation to 10% (2 of 19) of the

**Table 1.** Influence of lymphocyte transfer on diabetes incidence in diabetes-prone BB/OK rats

Parameter	Dimension	Sham-transferred BB/OK rats	Adoptively-transferred BB/OK rats
Diabetes Incidence	%	33.3 (7/21)	10.5 (2/19)
Sex		4 male; 3 female	2 female
Age at onset	Days	$109 \pm 3$	$152 \pm 41$

treated BB/OK rats and modified the time of manifestation in one of the two diabetic animals.

The islet insulin content of the 2000 grafted islets did not differ significantly between the several groups investigated and remained between  $9020 \pm 1280$  pmol and  $11500 \pm 440$  pmol.

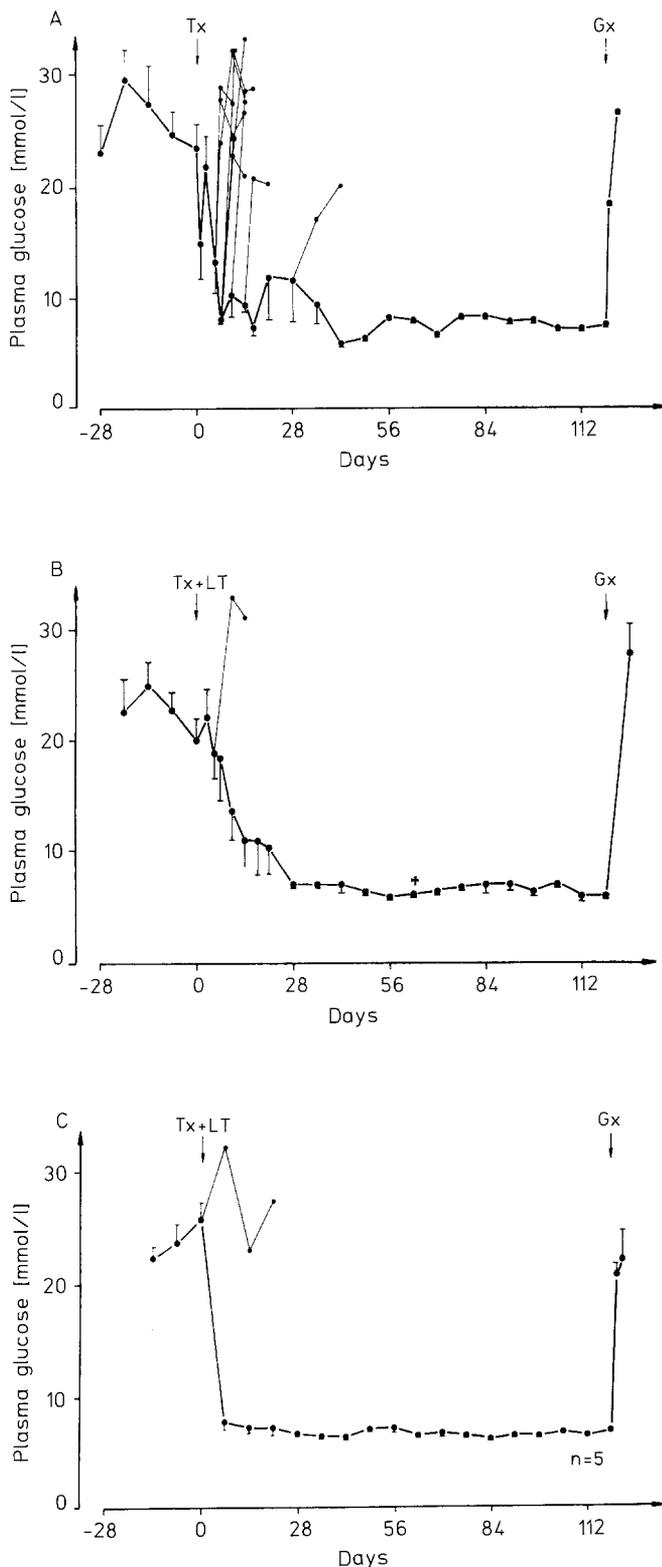
Following transplantation of the BB/OK rat islets into untreated diabetic BB/OK rats, we observed a graft destruction in 10 of 12 recipients (mean survival time  $> 28.5 \pm 12.5$  days) by the recurrence of hyperglycaemia within 20 days (Fig. 1 A). When the transplantation was combined with an additional transfusion of lymph node and spleen cells prepared from long-term normoglycaemic BB/OK rats, normoglycaemia was reached within 4 weeks in 9 of 10 animals (Fig. 1 B) and was maintained for at least 120 days (mean survival time  $> 97.1 \pm 12.0$  days). As Figure 1 C demonstrates, peripheral blood lymphocytes also prevented graft destruction in five of six islet grafted BB/OK rats for 120 days (mean survival time  $101.0 \pm 19.0$  days). The graft removal caused an immediated increase in plasma glucose, indicating that the maintained normoglycaemia was the result of a viable islet graft (Figs. 1 B, 1 C). The graft insulin content at day 120 amounted to  $17116 \pm 2101$  pmol in the BB/OK rats which were transfused with lymph node and spleen cells. The pancreatic insulin content remained low in each situation investigated.

The lymphocyte number transferred into the diabetic recipients of the two islet grafted groups (see Materials and methods) was significantly different ( $p < 0.01$ ).

The transfer of lymph node and spleen cells did not prevent the destruction of pancreatic islets obtained from third party LEW.1A donors when transplanted into diabetic BB/OK rats. The mean survival time in such animals amounted to  $14.2 \pm 2.6$  days ( $n = 6$ ) which is identical to untreated BB/OK rats grafted with allogeneic islets ( $14.4 \pm 1.6$ ,  $n = 10$ ).

## Discussion

There is increasing evidence that insulin-dependent diabetes mellitus in humans and certain animal models (BB rat, NOD mouse) is autoimmune in nature [12, 13]. A cell-mediated autoimmune pancreatic Beta-cell destruction is discussed, because firstly, the disease can be transferred by lymphocytes [14–16], and secondly, thymectomy [17, 18], immunosuppression and immunomodulation prevent onset of disease [19–22]. The islet destruction process leading to hyperglycaemia has a long period before the onset of disease, in which phases of active destruction probably alternate with phases of a suppressed immune attack. Prior to diabetes onset a reduction of pancreatic Beta-cell volume and the appearance of insulinitis was observed in the animals [2]. This was also observed in those animals which never developed diabetes [2, 23], supporting the hypothesis of a spontaneous cessation of autoaggression in these animals. The described suppression of autoimmune pancreatic Beta-cell destruction by lymphocytes obtained from long-term normoglycaemic BB/OK rats in which an immune attack had occurred in different



**Fig. 1A-C.** Plasma glucose of autoimmune diabetic BB/OK rats after syngeneic islet transplantation with and without transfusion of lymphocytes obtained from long-term normoglycaemic BB/OK rats. **A** Islet transplantation without treatment of recipients (control);  $n = 12$ . **B** Islet transplantation in combination with a transfusion of lymphocytes prepared from cervical lymph nodes and spleen;  $n = 10$ . **C** Islet transplantation in combination with a transfusion of lymphocytes prepared from peripheral blood;  $n = 6$ . Tx = Islet transplantation; LT = Lymphocyte transfer; G<sub>x</sub> = Graft removal; † One animal died under anaesthesia

experimental series suggests that a cell-mediated mechanism was responsible for the maintenance of normoglycaemia in the lymphocyte donors. Neither the lymphocyte number nor the cell composition of the lymphocytes transferred (data not shown) allows any conclusion regarding the cell population(s) involved. It seemed likely that T lymphocyte subpopulations were responsible for the observed effect [15].

In contrast to Rossini et al. [15, 24] and Logothetopoulos et al. [25] but in agreement with Burstein et al. [26] we prevented diabetes development by a single transfusion of lymphocytes. The cited authors [15, 25, 26] used lymphocytes obtained from immunologically normal rat strains (diabetes-resistant, RT6<sup>+</sup>) to arrest the autoimmune Beta-cell destruction, whereas in our study long-term normoglycaemic animals (in which insulinitis had occurred) of a diabetes-prone BB rat strain served as lymphocyte donors.

Greiner et al. [27] observed diabetes development in BB rats only in the absence of a special lymphocyte population (RT6<sup>+</sup> cells). The successful transfer of lymphocyte containing RT6<sup>+</sup> cells into RT6<sup>-</sup> diabetes-prone BB rats and the detection of the missing cell population following the transfer suggested that the prevention of diabetes may be mediated by RT6<sup>+</sup> lymphocytes [26]. Our results, however, do not confirm a crucial role of RT6<sup>+</sup> T-lymphocytes in preventing diabetes. On the contrary, our experiments clearly demonstrated the efficacy of an RT6<sup>-</sup> lymphocyte population, because both the lymphocyte donors and recipients belong to the BB/OK rat strain which is negative for RT6 T cells [28]. The third party transplantation demonstrated as expected, that the cell-mediated mechanism found in long-term normoglycaemic BB/OK rats prevented autoimmune destruction of pancreatic Beta cells but not the allogeneic rejection.

In summary, the mechanism responsible for maintaining normoglycaemia in long-term normoglycaemic BB/OK rats is cell-mediated, and can probably be maintained for life, indicating a possible role of memory cells. Furthermore, this property can be transferred to prevent autoimmune destruction of pancreatic Beta cells.

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