

Originals

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

B. Kuttler¹, A. Dunger¹, H. D. Volk², T. Diamantstein³ and H. J. Hahn¹

¹ Institute of Diabetes "G. Katsch", Karlsburg, ² Institute of Medical Immunology, Charite, Humboldt-University, and

³ Institute of Immunology, Klinikum Steglitz, Free University, Berlin, FRG

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 ± 0.2 mmol/l) at an age of 92 ± 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 ± 0.1 mmol/l) with an age of 232 ± 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 ± 3 | 152 ± 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 ± 1280 pmol and 11500 ± 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 ± 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 ± 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 ± 2.6 days ($n = 6$) which is identical to untreated BB/OK rats grafted with allogeneic islets (14.4 ± 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

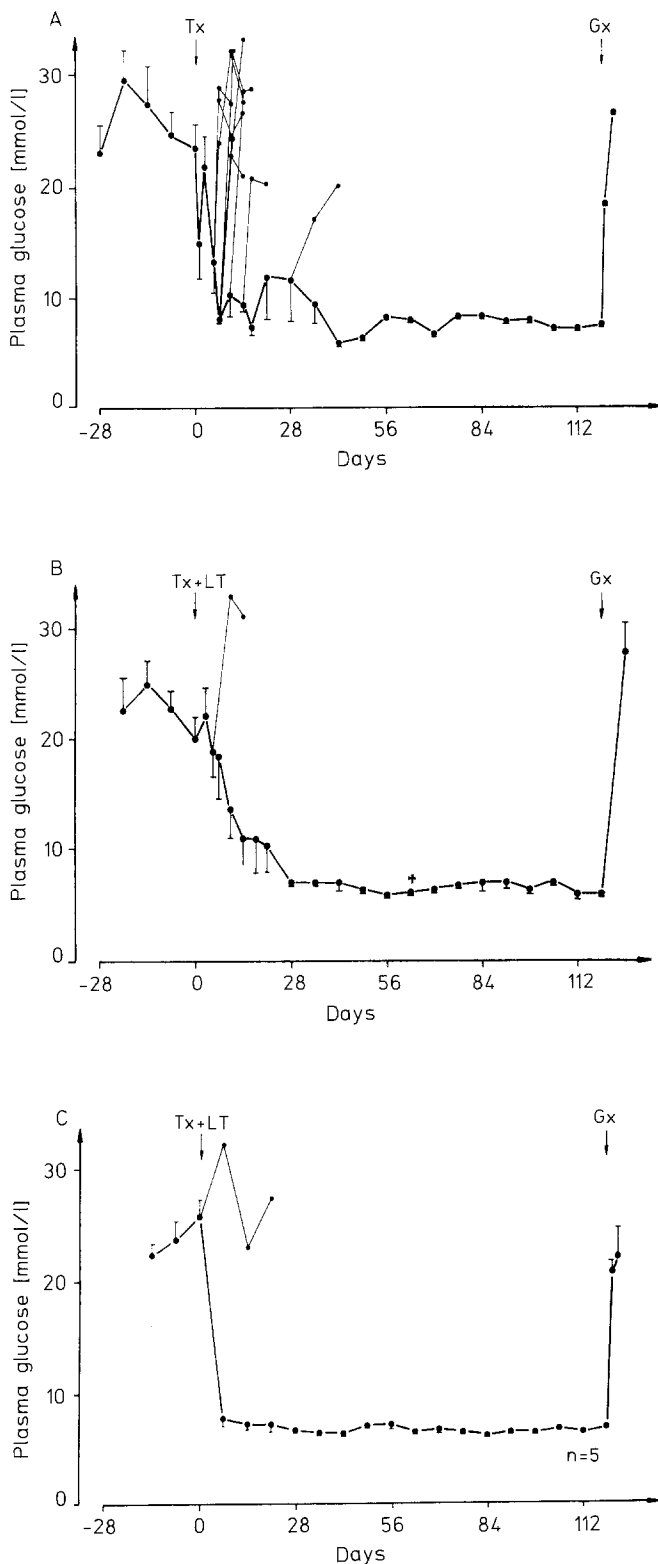


Fig. 1A–C. Plasma glucose of autoimmune diabetic BB/OK rats after syngeneic islet transplantation with and without transfusion of lymphocytes obtained from long-term normoglycaemic BB/OK rats. **A** Islet transplantation without treatment of recipients (control); $n = 12$. **B** Islet transplantation 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_x = 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⁺) 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⁺ cells). The successful transfer of lymphocyte containing RT6⁺ cells into RT6⁻ 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⁺ lymphocytes [26]. Our results, however, do not confirm a crucial role of RT6⁺ T-lymphocytes in preventing diabetes. On the contrary, our experiments clearly demonstrated the efficacy of an RT6⁻ 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.

Acknowledgements. This study was part of the research project HFR 29 "Organtransplantation und Gewebekonservierung" of the Ministry of Health of the former GDR. The authors would like to thank Ms. Ch. Kauert, Ms. K. Gumm, Ms. H. Ohlrich, Ms. G. Strauch, Ms. M. Henkel, Ms. M. Behm and Ms. S. Kiowski for technical assistance and Ms. P. Schultz for typing the manuscript.

References

1. Naji A, Silvers WK, Barker CF (1985) Cell-mediated immunity in type I (insulin-dependent) diabetes of man and the BB rat. In: Cruse JM, Lewis RE (eds) Concepts immunopathol, Vol 2. Karger, Basel, pp 32–46
2. Lucke S, Diamantstein T, Hahn HJ (1989) Different lymphocyte subset distribution within "insulinitis" islets of normoglycaemic and prediabetic BB/OK rats of similar age. *Exp Clin Endocrinol* 94: 57–63
3. Weringer EJ, Like AA (1985) Immune attack on pancreatic islet transplants in the spontaneously diabetic Bio Breeding/Worcester (BB/W) rat is not MHC restricted. *J Immunol* 134: 2283–2286

4. Weringer EJ, Like AA (1986) Diabetes mellitus in the BB/W rat: Insulinitis in pancreatic islet grafts after transplantation into diabetic recipients. *Am J Pathol* 125: 107–112
5. Naji A, Silvers WK, Bellgrau D, Anderson AO, Plotkin S, Barker CF (1981) Prevention of diabetes in rats by bone marrow transplantation. *Ann Surg* 194: 328–338
6. Naji A, Bellgrau D, Anderson A, Silvers WK, Barker CF (1982) Transplantation of islets and bone marrow cells to animals with immune insulinitis. *Diabetes* 31 [Suppl 4]: 84–89
7. Klötting I, Reiher K (1985) Einige Aspekte zur Haltung und Reproduktion spontandiabetischer BB Ratten. *Z Versuchstierk* 27: 5–11
8. Kuttler B, Volk HD, Kauert Ch, Diamantstein T, Hahn HJ (1989) Phenotyping of lymphocytes following transplantation of allogeneic rat pancreatic islets into streptozotocin-diabetic recipients. *Exp Clin Endocrinol* 94: 64–69
9. Hehmke B, Kohnert KD, Odselius P (1986) The use of a new dextran gradient for rapid isolation of functionally intact neonatal pancreatic islets. *Diabetes Res* 3: 13–16
10. Hahn HJ (1978) Die isolierte Langerhanssche Insel, ein Modell zur Untersuchung der Insulinsekretion in vitro. *Endokrinologie* 71: 308–324
11. Ziegler B, Hahn HJ, Ziegler M (1985) Insulin recovery in pancreas and host organs of islet grafts. *Exp Clin Endocrinol* 85: 53–60
12. Cahill GF Jr, McDevitt HO (1981) Insulin-dependent diabetes mellitus: the initial lesion. *N Engl J Med* 304: 1454–1465
13. Rossini AA, Mordes JP, Like AA (1985) Immunology of insulin-dependent diabetes mellitus. *Ann Rev Immunol* 3: 291–322
14. Like AA, Weringer EJ, Holdash A, McGill P, Atkinson D, Rossini AA (1985) Adoptive transfer of autoimmune diabetes mellitus in Biobreeding/Worcester (BB/W) inbred and hybrid rats. *J Immunol* 134: 1583–1585
15. Rossini AA, Faustman D, Woda BA, Like AA, Szymanski J, Mordes JP (1984) Lymphocyte transfusions prevent diabetes in the Bio-Breeding Worcester rat. *J Clin Invest* 74: 39–46
16. Wicker LS, Miller BJ, Mullen Y (1986) Transfer of autoimmune diabetes mellitus with splenocytes from nonobese diabetic (NOD) mice. *Diabetes* 35: 855–860
17. Like AA, Kislauskis E, Williams RM, Rossini AA (1982) Neonatal thymectomy prevents spontaneous diabetes mellitus in the BB/W rat. *Science* 216: 644–646
18. Ogawa M, Maruyama T, Hasegawa T, Kanaya T, Kobayashi F, Tochino Y, Uda H (1985) The inhibitory effect of neonatal thymectomy on the incidence of insulinitis in nonobese-diabetic (NOD) mice. *Biomed Res* 6: 103–105
19. Laupacis A, Stiller CR, Gardell C, Keown P, Dupre J, Wallace AC, Thibert P (1983) Cyclosporin prevents diabetes in BB Wistar rats. *Lancet* I: 10–12
20. Mori Y, Suko M, Okudeira H, Matsuba J, Tsuruoka A, Sasaki A, Yokoyama H, Tanase T, Shida T, Nishimura M, Terada E, Ikeda Y (1986) Preventive effects of cyclosporin on diabetes in NOD mice. *Diabetologia* 29: 244–247
21. Hahn HJ, Lucke S, Klötting I, Volk HD, v Baehr R, Diamantstein T (1987) Curing BB rats of freshly manifested diabetes by short-term treatment with a combination of monoclonal anti-interleukin 2 receptor antibody and a subtherapeutic dose of cyclosporine A. *Eur J Immunol* 17: 1075–1078
22. Like AA, Biron CA, Weringer EJ, Byman K, Sroczynski E, Guberski DL (1986) Prevention of diabetes in Bio-Breeding/Worcester rats with monoclonal antibodies that recognize T lymphocytes or natural killer cells. *J Exp Med* 164: 1145–1159
23. Lee KU, Amano K, Yoon JW (1988) Evidence for initial involvement of macrophage in development of insulinitis in NOD mice. *Diabetes* 37: 989–991
24. Rossini AA, Mordes JP, Pelletier AM, Like AA (1983) Transfusions of whole blood prevent spontaneous diabetes in the BB/W rat. *Science* 219: 975–977
25. Logothetopoulos J, Shimak K, Baily D (1988) Prevention of spontaneous but not adoptively transferred diabetes by injection of neonatal BB/hooded hybrid rats with splenocytes or Concanavalin A blasts from diabetes-free strains. *Diabetes* 37: 1009–1014
26. Burstein D, Mordes JP, Greiner DL, Stein D, Nakamura N, Handler ES, Rossini AA (1989) Prevention of diabetes in BB/Wor rats by single transfusion of spleen cells. Parameters that affect degree of protection. *Diabetes* 38: 24–30
27. Greiner DL, Handler ES, Nakano K, Mordes JP, Rossini AA (1986) Absence of RT-6 T cell subset in diabetes-prone BB/W rats. *J Immunol* 136: 148–151
28. Klötting I, Vogt L, Günther E, Wurst W, Hedrich HJ, Reetz JC (1989) Availability of newly established congenic rat strains LEW.1BB and BB.1A. *RNL* 21: 22–23

Received: 8 February 1990
and in revised form: 15 August 1990

Dr. B. Kuttler
Institute of Diabetes "G. Katsch"
O-2201 Karlsburg
FRG