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Prolongation of rat pancreatic islet allograft survival by treatment of recipient rats with monoclonal anti-interleukin-2 receptor antibody and cyclosporin

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Summary. Since interleukin-2-receptor expressing cells play a role in allograft rejection, we investigated the effect of anti-interleukin-2 receptor monoclonal antibody treatment on graft survival of allografted pancreatic islets. When pancreatic islets obtained from Lewis A-rats (haplotype RT1^a) were grafted under the kidney capsules of streptozotocin-diabetic Lewis rats (haplotype RT1^u), the recipients relapsed into hyperglycaemia within 11 days (7 ± 1 days). Treatment of the recipient rats with low-dose cyclosporin (1.5 mg/kg body weight) had no effect on allograft survival (9 ± 1 days). The application of anti-interleukin-2 receptor monoclonal antibody (1 mg/kg body weight) for 10 days resulted in a prolongation of allograft survival (42.5 ± 15.3, $p < 0.01$). In 3 out of 11 animals a permanent normoglycaemia (> 120 days) associated with glucose intolerance was observed. When the recipients were treated for 10 days with cyclosporin and anti-interleukin-2 receptor monoclonal antibody, the allograft survival was also prolonged (45.1 ± 14.6, $p < 0.01$); again 3 out of 11 animals remained permanently normoglycaemic while exhibiting a normal glucose tolerance.

Key words: Pancreatic islet allograft, immunotherapy, anti-IL-2 RMAB, cyclosporin, graft histology.

Prevention and treatment of allograft rejection remain the major problems in clinical organ transplantation. It is generally accepted that sensitized T lymphocytes play a pivotal role in allograft rejection. Sensitized T cells express transiently the receptor for interleukin 2 (IL-2), the major cytotrophic factor for immunocompetent T lymphocytes [1]. The interaction of IL-2 with receptor bearing cells is a prerequisite for clonal expression and continued viability of activated T cells. Because all proliferating T lymphocytes express IL-2 receptors, a receptor-targeted therapy should create a selective immune defect in the recipient [1]. Very recently Lord et al. [2] demonstrated the existence of IL-2 receptor bearing cells in infiltrated cardiac allografts during rejection. Recipients' treatment with IL-2 receptor antibodies leads to a prolongation of allografted hearts [3].

In this study we investigated the application of a mouse monoclonal antibody on allogeneic rejection in rat pancreatic islet transplantation. The monoclonal antibody used (ART 18 mab) reacts with the rat lymphoblasts, identifies the rat IL-2 receptor and inhibits binding of IL-2 to IL-2 receptor (IL-2R) positive cells as well as IL-2 dependent proliferation of IL-2R bearing cells [1, 4].

Materials and methods

Animals

Female Lewis rats (LEW.1W MaxK, haplotype RT1^u) with a body weight of 180 g, made diabetic by application of streptozotocin (50 mg/kg body weight, Upjohn Company Kalamazoo, Mich, USA), served as recipients. About 10 days after induction of hyperglycaemia the rats were anaesthesized, the abdominal cavity opened and the pancreas biopsied to measure pancreatic insulin content [5]. One thousand isolated islets obtained from LEW.1A MaxK rats (haplotype RT1^a) were grafted under the right kidney capsules.

Body weight and plasma glucose were measured daily for the first 3 weeks, then 3 times weekly up to week 7 and weekly up to week 17. Graft rejection was established when a glucose concentration above 9.5 mmol/l (+2 SD above normal range of LEW.1W MaxK rats) was determined by 2 consecutive measurements within 3 days.

The hyperglycaemic animals were killed 1 week after rejection, the kidney removed for morphological investigation and the pancreatic insulin content measured [5]. In normoglycaemic rats, an intraperitoneal glucose tolerance test was performed 40 and 120 days after transplantation. One day later a pancreatic biopsy was taken to repeat the determination of pancreatic insulin content [5]. During the last operation the right kidney was removed for histological investigation. After graft removal, plasma glucose was measured daily for 4 days.

Islet isolation and graft characterisation

Pancreatic islets were prepared from 8- to 12-day-old LEW.1A MaxK rats using a fractionated collagenase digestion. About 1050 freshly

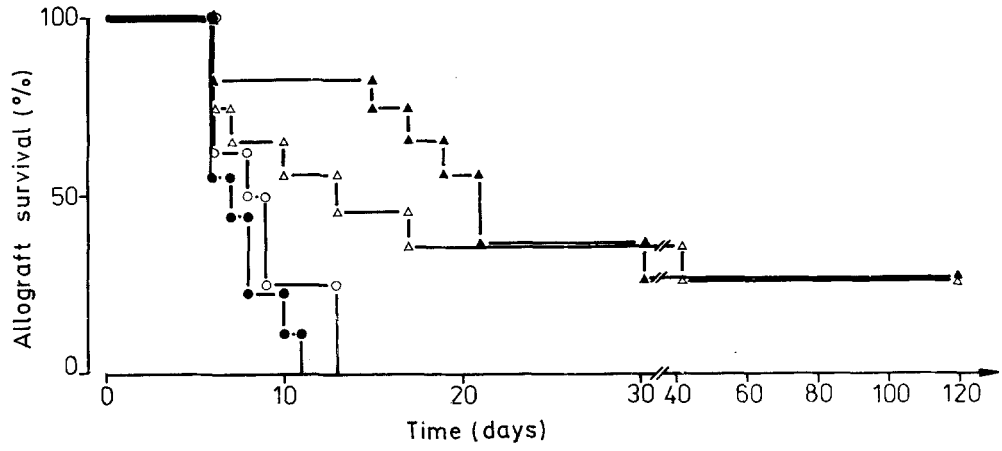

Fig. 1. Allograft survival rate (given in % of all animals investigated in the respective group) of untreated (●—●, $n=9$), cyclosporin-treated (○—○, $n=9$) and anti-IL-2 receptor mab-treated (△—△, $n=11$) recipients. Eleven animals (▲—▲) were treated with anti-IL-2 receptor mab and cyclosporin. The treatment period was 10 days

isolated islets were collected, and 50 islets were taken for graft characterization. Islet insulin content was measured after ultrasonic homogenization in triplicates (3 times 5 islets) [6]. After a preincubation period of 30 min, 10 islets were incubated for 120 min in a modified Krebs-Ringer bicarbonate buffer at 37 °C in an atmosphere of 95% O_2 and 5% CO_2 (pH 7.4) in the presence of 1.5 or 20.0 mmol/l glucose [6].

Immunotherapy

The grafted animals were either used as untreated control rats (group 1, $n=9$) or treated daily with cyclosporin (group 2, 1.5 mg/kg body weight intramuscularly for 10 days, $n=9$, Sandoz AG, Basel, Switzerland), or anti-IL-2 RMAB (group 3, 1 mg/kg body weight intraperitoneally for 10 days, $n=11$). Finally, a group of animals (group 4, $n=11$) was treated for 10 days with cyclosporin and anti-IL-2 RMAB. The production and biological characterization of the anti-interleukin-2-receptor monoclonal antibody (anti-IL-2R-mab) used has been published elsewhere [1, 4].

Morphology

The removed kidneys were fixed in Bouin's solution, embedded in paraffin and cut in 7 µm serial sections, which were either stained with haematoxylin-eosin or immunostained for insulin using a monoclonal anti-insulin antibody (IAK-36a C10 mab) and fluorescein-isothiocyanat-labelled anti-mouse immunoglobulins (Staatliches Institut für Immunpräparate und Nährmedien, Berlin, GDR) [7].

Analytical methods

Plasma glucose taken from the tail vein was measured by using a Beckman glucose analyzer (Beckman Instruments, Fullerton, Calif, USA). To assess the animals' glucose tolerance, 2.0 g glucose were applied per kilogramme body weight intraperitoneally, and plasma glucose was determined at 0, 10, 30, 60 and 120 min. The insulin concentrations in tissue and buffer were measured by radioimmunoassay.

Statistical analysis

The results were calculated as mean ± SEM of n different animals. Statistical significance was evaluated by Student's t-test or by the Wilcoxon or Mann and Whitney's U-test (graft survial). A p value of <0.01 was considered statistically significant. The glucose tolerance is given as integrated area under the glucose curve during the test period of 120 min. The graft survival is given in percent of the total group of animals transplanted.

Results

At the time of transplantation the grafted animals had a marked hyperglycaemia, which did not differ between the groups (ranging between 28.6 ± 1.8 mmol/l (group 4) and 29.7 ± 1.2 mmol/l (group 3)), and a markedly reduced pancreatic insulin content (ranging between 0.73 ± 0.14 pmol/mg wet weight (group 4) and 1.38 ± 0.47 pmol/mg (group 1)). The different islet preparations used for transplantation did not differ in their islet insulin content (ranging between 6.31 ± 0.48 pmol/islet (group 1) and 6.99 ± 0.37 pmol/islet (group 2)), and were characterized by a markedly enhanced insulin secretion in response to glucose (data not shown). The grafted untreated animals (group 1) relapsed into hyperglycaemia within 7.3 ± 0.5 days. The application of 1.5 mg cyclosporin/kg body weight (group 2) did not result in a significant prolongation of the mean graft survival time (8.8 ± 1.0 days) (Fig. 1). Seven days after the onset of rejection, the plasma glucose was above 25 mmol/l in both groups.

Treating the recipients with 1 mg anti-IL-2 RMAB/kg body weight for 10 days (group 3) resulted in a significant (42.5 ± 15.3, $p<0.01$) prolongation of graft survival, and in 3 out of 11 animals a permanent graft acceptance occured (Fig. 1). The mean non-fasting plasma glucose of the 3 long-term acceptors (weekly determination from days 42 to 119 after transplantation) was 8.92 ± 2.41 mmol/l (n = 36). The glucose tolerance at day 40 was 1934 ± 438 $mmol \cdot l^{-1} \cdot min^{-1}$ and at day 120 1769 ± 357 $mmol \cdot l^{-1} \cdot min^{-1}$. These animals developed marked hyperglycaemia immediatedly after graft removal. On histology the grafts were virtually free of lymphocytic infiltrations, and the B cells contained insulin as demonstrated by immunohistochemistry (data not shown).

The combined application of cyclosporin and anti-IL-2Rmab (group 4) resulted in a significantly prolonged graft survival (45.1 ± 14.6 days, $p<0.01$, versus the untreated animals of group 1), and in 3 out of 11 animals a permanent graft acceptance occured (Fig. 1). The

mean non-fasting plasma glucose (determined between days 42 and 119) in these three animals was 6.18 ± 0.71 mmol/l ($n = 36$), and the glucose tolerance was 1142 ± 21 mmol·l^{-1}·min^{-1} at day 40 and 1351 ± 43 mmol·l^{-1}·min^{-1} at day 120 (normal range ± 2 SD $= 1107 - 1571$ mmol·l^{-1}·min^{-1}). In these animals graft removal resulted in the immediate development of hyperglycaemia, since the pancreatic insulin content was still very low (0.78 ± 0.08 pmol/mg). Graft morphology demonstrated a tissue free of infiltrating cells with well granulated pancreatic B cells as demonstrated by immunocytochemistry (data not shown).

Discussion

Since the diabetic recipients, characterized by the determination of hyperglycaemia and pancreatic insulin content, and the graft viability and function, characterized by determination of islet insulin content and insulin secretion in vitro, were identical at transplantation, the observed differences of mean survival time can only be ascribed to the different treatment of the recipient rats. In accordance with earlier studies from this laboratory [8], grafted pancreatic islets were rejected within 11 days after transplantation, indicating that the selected histocompatibility barrier could not be overcome if freshly isolated pancreatic islets were used as donor tissue, and if no immunosuppression was induced. Whereas low dose cyclosporin treatment was ineffective in preventing graft rejection, the temporary application of anti-IL-2R mab resulted in a prolongation of islet survival when applied alone and also when given together with low dose cyclosporin, suggesting that IL-2 expressing cells are involved in graft rejection [2]. The anti-IL-2R mab therapy may exert its effects in vivo either by blocking the IL-2 receptor [1, 4], thereby inhibiting the clonal expansion of the antigen-activated cells, or alternatively by eliminating the IL-2R bearing cells. Recently, it has been shown that temporary treatment of allografted heart recipients, with mab directed to the IL-2 receptor, resulted in a postponement of allograft rejection [3]. In contrast to the results observed in experimental heart transplantation, we observed under respective experimental conditions in 3 out of 11 animals a permanent pancreatic islet graft survival (> 120 days). The marked glucose intolerance observed in these 3 rats after transplantation of 1000 pancreatic islets (which corresponds to about 35% of total pancreatic insulin content of adult rats) led us to assume that a considerable part of the transplanted islets must have been destroyed under these conditions. The additional application of cyclosporin together with anti-IL-2Rmab resulted also in a permanent acceptance in 3 out of 11 rats. Even this combined form of therapy did not, however, prevent allograft rejection. Further studies with modified doses, treatment periods and modulated pancreatic islets [9] might lead to a further increase of graft acceptance rates.

Nevertheless, this study demonstrated that the 3 animals with long-term acceptance of the allograft after combined treatment of cyclosporin and anti-IL-2 RMAB maintained not only normoglycaemia, but also a normal glucose tolerance up to 120 days, possibly suggesting a synergistic effect of both immunomodulators. A similar synergistic effect of cyclosporin and anti-IL-2Rmab could also be recently observed in heart allografts [10]. In conclusion, the results demonstrated an inhibitory action of anti-IL-2R mab on graft rejection, even though the mab was only temporarily administered.

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