*Rapid communications***Effects of ganglioside (Cronassial) treatment on MHC Ia antigen expression and allograft survival of pancreatic islets in diabetic rats**

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Summary. Streptozotocin-diabetic BdII rats were treated daily with 20 mg/kg body weight gangliosides for ten days beginning two days before transplantation. This treatment did not prolong allograft survival of untreated Lewis islets. Culture treatment of isolated Lewis islets with gangliosides (100 µg/ml in RPMI 1640) for one day resulted in a significant reduction of MHC Ia antigen positive cells but not of

class I antigens within the islets. Transplantation of the ganglioside pretreated islets into non-immunosuppressed BdII recipients prolonged allograft survival to 12 days only in one of five animals.

Key words: Gangliosides, islet transplantation, immunosuppression, immunomodulation.

Gangliosides are natural components of human and animal plasma membranes [1]. Ganglioside preparations are used in the therapy of peripheral neuropathies such as the diabetic neuropathy [2].

There are also reports on the influence of gangliosides on the immune response. The *in vitro* activation of human lymphocytes by nonspecific mitogens and allogeneic cells [3] is inhibited by gangliosides inducing a rapid and selective disappearance of the CD4 molecules from T helper cells [4]. The molecular change of CD4 in the T helper cell membrane prevents antibody binding. In addition, an inhibition of interleukin-2-dependent cytotoxic T lymphocyte growth by gangliosides was reported [5].

As CD4 (L3T4) positive T helper cells are known to be required to induce insulinitis and diabetes in neonatal and healthy young NOD mice, the *in vivo* effect of gangliosides on the diabetes incidence of NOD mice and BB rats was tested [6, 7]. A significant reduction of diabetes incidence in NOD mice but not in BB rats was shown.

Because of the interaction of gangliosides with the immune system, we tested whether gangliosides have an immunosuppressive or an immunomodulative effect on islet allo-transplantation in streptozotocin-diabetic rats.

Materials and methods*Animals*

Male Lewis rats (RT1^l) of about 250 g (Savo Ivanovas GmbH, Kisslegg, FRG) were used as islet donors. Male BdII rats (RT1ⁿ) of about 250 g (Tierzuchtinstitut, Hannover, FRG) and male Lewis rats as control were made diabetic by a single *i. v.* injection of streptozotocin (55 mg/kg body weight; Upjohn, Kalamazoo, Mich., USA).

Gangliosides

Gangliosides (Cronassial) were kindly donated by Dr. Romandini, (Fidia GmbH, München, FRG), as pure substance containing 21% G_{M1}, 40% G_{D1a}, 16% G_{D1b}, 19% G_{T1b}, and 5% other gangliosides.

Islet isolation

The islets of the Lewis rats were isolated by ductal collagenase (Serva, Heidelberg, FRG) distension of the pancreas followed by incubation at 37°C. Purification was done by Ficoll (Ficoll 400; Sigma, Deisenhofen, FRG) density gradient centrifugation [8]. Remaining exocrine tissue was removed by handpicking under a dissecting microscope.

Islet pretreatment

For pretreatment with gangliosides the islets were incubated in RPMI 1640 Medium (Gibco, Eggenstein, FRG), containing 100 µg/ml Cronassial, 10% fetal calf serum (Gibco), 1% penicillin (5000 IU/ml), streptomycin (5000 µU/ml, Flow Laboratories, Neckenheim, FRG), and 1% L-glutamine (200 mmol/l, Flow Laboratories) for 24 h at 37°C in 5% CO₂ and 95% humidified air. Islets treated in that way were either used for transplantation or for MHC Ia antigen detection *in vitro*. Untreated islets were used as controls after culture for 24 h.

Islet transplantation

For allo-transplantation studies diabetic BdII rats and Lewis rats for syngeneic transplantations (blood glucose 16.8 mmol/l for two weeks) were anaesthetised by ether. About 1000 ganglioside-treated or untreated islets per recipient were transplanted into the portal

vein. Fasting blood glucose was measured every morning and rejection was defined by blood glucose > 11.2 mmol/l.

One group of the diabetic BdlI recipients was treated daily with gangliosides beginning two days prior to and up to eight days following transplantation. Gangliosides were given s.c. in a dose of 20 mg/kg body weight dissolved in isotonic NaCl solution.

Immunofluorescence studies

MHC antigen detection was done on whole islets by indirect immunofluorescence tests. Untreated islets or islets pretreated with gangliosides were incubated with the monoclonal antibodies OX-6 (directed against rat Ia antigens; Serotec, Oxford, UK) or OX-18 (directed against rat class I antigens; Serotec) for 30 min at 4°C. After washing, fluorescein isothiocyanate-conjugated rabbit anti mouse immunoglobulin (Dakopatts, Hamburg, FRG) was added. Washed islets were transferred onto microscopic slides and in each assay 100 islets were examined for the number of Ia antigen positive cells per islet and for class I antigen expression under a fluorescence microscope (490 nm).

Statistical analysis

Results are expressed as mean $\pm$ SEM. For statistical analysis the parameter free U test of Mann and Whitney was used. Significance was defined as $\alpha < 0.1\%$.

Results

Islet transplantation

We could not show any significant immunosuppressive effect of the gangliosides. Treatment of the islet recipients with gangliosides for ten days beginning at day 2 before transplantation did not prolong allograft survival (Table 1). There were no significant differences between the allograft rejection in untreated and in ganglioside-treated islet recipients (Table 1).

Using gangliosides as an immunomodulative substance we found that pretreatment of the islets with gangliosides for one day prolonged the allograft acceptance in non-immunosuppressed recipients up to 12 days only in one of five animals (Table 1). In the other cases the islets were rejected after three or four days.

As a control for the function of untreated islets, we syngeneically transplanted Lewis islets into untreated Lewis rats. Survival times of the isografts were > 100 days in all of the diabetic recipients.

Indirect immunofluorescence

Islet incubation with gangliosides for 24 h had a beneficial effect on morphological islet integrity after isolation as compared to freshly isolated islets. The islets were round shaped and had a smooth pseudomembrane-like surface.

Indirect immunofluorescence revealed that pretreatment of the islets with gangliosides caused an evident reduction of Ia antigen positive cells within the islets. $76.3 \pm 3.1\%$ of the islets pretreated with gangliosides were

Table 1. Influence of recipient and islet pretreatment with gangliosides on islet allograft survival. For comparison the results of islet isografts are given. Each group consisted of five animals

Donor	Recipient	Islet pretreatment	Treatment of the recipient	Days of normoglycaemia
Lewis	BdlI	none	10 day ganglioside	3, 3, 4, 4, 4
Lewis	BdlI	1 day ganglioside	none	3, 3, 4, 4, 12
Lewis	BdlI	none	none	3, 4, 4, 5, 5
Lewis	Lewis	none	none	$5 \times > 100$

completely negative for Ia antigen positive cells compared to $6.5 \pm 2.2\%$ of the untreated islets (significance, $\alpha < 0.1$). In addition, ganglioside pretreatment decreased the maximum number of Ia antigen positive cells per islet from about 40 to about 10.

On the contrary, gangliosides did not have any effect on class I antigen expression. The vascular endothelial cells were stained very intensively, whereas the other cells showed little fluorescence in treated as well as in untreated islets.

Discussion

Gangliosides can inhibit the in vitro lymphocyte activation mediated by nonspecific mitogens and allogeneic cells [3]. Pretreatment of T helper lymphocytes with gangliosides induces a rapid and selective disappearance of the CD4 molecules from the T cells [4]. In vitro studies also revealed an inhibition of interleukin-2-dependent cytotoxic T lymphocyte growth by gangliosides [5]. These immunological properties of gangliosides were used to reduce diabetes incidence in NOD mice [7], probably induced by an infiltration with CD4 (L3T4) positive T-cells.

We tried to clarify whether gangliosides also can impair the T-cell response in islet allograft rejection and can thus be used as an immunosuppressive agent. However, we could not find any support for this hypothesis. There were no differences in the allograft survival time between animals treated with gangliosides and untreated animals. These findings may be explained by the fact that already 10% serum completely neutralizes the ganglioside effect on CD4 expression [4], thus leading to the controversial in vivo and in vitro results.

Pretreatment of the islets with gangliosides for 24 h altered the islet surface which appeared smooth as usually seen in islets cultured for ten days. Gangliosides are probably embodied into the membranes of the islet cells. This process seems to cause a change in the MHC Ia antigen expression. Ganglioside pretreatment induced a marked reduction of the number of Ia antigen positive islet cells. The mechanism of the evident antigen reduction remains unclear. On the contrary, class I antigen expression was not impaired by ganglioside pretreatment.

Despite the observed significant Ia antigen reduction a prolonged islet allograft survival from four to 12 days was seen only in one of five non-immunosuppressed animals.

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