Rapid communications

Pathogenesis of low dose streptozotocin induced diabetes in mice: requirement for $\alpha 1$ -adrenoceptor activation and vasoactive amine release

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Summary. Pancreatic islet inflammation and subsequent diabetes was induced by multiple low doses of streptozotocin in male C57 Bl/6J mice. The development of hyperglycaemia was almost completely prevented by treating the animals with the $\alpha 1$ -adrenoceptor antagonist prazosin ($20 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) as well as by the vasoactive amine antagonists methysergide ($50 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$), disodium cromoglycate ($100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$), pizotifen ($5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) or cyproheptadine ($20 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$). Treatment with vasoactive amine antagonists largely inhibited infiltration of pancre-

atic islets by L3T4⁺-lymphocytes and to a lesser extent by Lyt2⁺-cells. The infiltration of macrophages was not affected except after pizotifen treatment. These results indicate that $\alpha 1$ -adrenoceptor activation is required for disease development and that vasoactive amine release is a prerequisite for lymphocytic insulinitis but not for macrophage infiltration of islets.

Key words: Streptozotocin (low dose), prazosin, vasoactive amine antagonists, insulinitis.

Multiple low doses of streptozotocin induces in certain mouse strains inflammation of pancreatic islets and in part of the exocrine pancreatic tissue eventually resulting in insulin deficient diabetes [1, 2]. In general, the development of diabetes could be prevented by impairment of T-lymphocyte or macrophage function such as depletion of T-cells by monoclonal antibodies, treatment with silica particles or by using athymic nude mice [2].

Recently, it has been shown that streptozotocin administration causes oedema formation in islets [3, 4]. The vascular leakage in the islets could be inhibited by treating animals with methysergide, a blocker of the endothelial serotonin receptor. Concomitantly the development of hyperglycaemia was suppressed [4, 5].

In experimental autoimmune encephalitis it has been suggested that vascular leakage is preceded by vasoconstriction. We therefore studied whether vasoconstrictive events are involved in the disease process by *in-vivo* administration of prazosin, a specific $\alpha 1$ -adrenoceptor antagonist. Secondly, we analysed whether the blockade of vasoactive amines at various levels might affect lymphocyte and macrophage infiltration of islets.

Materials and methods

Low dose streptozotocin model

C57Bl/6J/Bom mice were obtained from Bomholtgard Ltd., (Ry, Denmark). The animals received water and food *ad libitum*. Diabetes was induced in 10-week old male mice by intraperitoneal injections of streptozotocin (Sz) (Boehringer, Mannheim, FRG) 40 mg/kg body weight on 5 consecutive days. Groups of 10–15 mice received either saline, prazosin or vasoactive drugs for 11 days starting 24 h after termination of Sz injections. The total daily dose (20 mg prazosin/kg body weight (Pfizer, Karlsruhe, FRG); 50 mg methysergide/kg (Sandoz, Nürnberg, FRG); 100 mg disodium-cromoglycate/kg (DSCG) (Fisons Arzneimittel GmbH, Köln, FRG); 5 mg pizotifen/kg (Sandoz); 20 mg cyproheptadine/kg (Merck Sharp & Dohme Research Lab, Rahway, NJ, USA)) was split into 5 parts and administered at 01.00, 06.30, 11.00, 15.00 and 19.30 hours. Blood glucose values and body weight were determined at days 0, 7, 14, 21, 30, 40, 50 and 60. The blood was obtained between 09.00 and 11.00 hours from the retro-orbital vein plexus of non-fasted mice and glucose concentrations were measured using the hexokinase method (Glucoquant, Boehringer, Mannheim, FRG).

Immunocytochemistry

For immunocytochemistry, pancreata were snapfrozen and frozen cut sections processed as described previously [6]. For this purpose we used rat monoclonal IgG2b antibodies to L3T4 (YTS191.1.2) and to

Fig. 1. Effect of prazosin, methysergide, DSCG, pizotifen and cyproheptadine on diabetes development: Mean blood glucose levels $\pm$ SEM. $\bullet$ -, Streptozotocin control; $\star$ -, prazosin treated mice; $\blacktriangle$ -, methysergide treated mice; $\blacksquare$ -, DSCG treated mice; $\square$ -, pizotifen treated mice; $\circ$ -, cyproheptadine treated mice

Lyt2 (YTS169.4.2) and rat monoclonal IgG2b antibody M1/70.15 (recognising 2 polypeptides on mouse macrophages and precursor cells) (Sera-lab, Paesel, Frankfurt, FRG). As second antibody rabbit-anti-rat IgG-biotin labelled (Vectastain ABC kit, Vector-Laboratories, Camon, Wiesbaden, FRG) was used.

Statistical analysis

Two investigators independently read randomly the mixed and coded slides. Statistical differences were calculated using Student's *t*-test (two tailed) for blood glucose levels and chi-square test for histological analysis. The level of significance was set at $p < 0.05$.

Results

Administration of low doses of streptozotocin on 5 consecutive days caused the development of diabetes by day 14 of the experiment (Fig. 1). Treatment with prazosin starting 24 h after the last streptozotocin injection almost completely prevented any rise in blood glucose levels throughout the observation period of 60 days. Blood glucose concentrations of normal nontreated animals of corresponding ages were in the range of 11 to 21 mmol/l. Mean values are shown in Figure 1. Differences between the prazosin and the streptozotocin control groups are significant ($p < 0.001$) at day 14 of the experiment and all later dates.

Four different antagonists of vasoactive amines were tested (methysergide, DSCG, pizotifen, cyprohep-

Table 1. Immunocytochemical analysis of pancreatic islets

Experimental group	Islets with immunocyte infiltration (%)		
	M1/70.15 ^a	L3T4 ^b	Lyt2 ^b
Streptozotocin + Saline	27 (n=33)	68 (n=25)	57 (n=23)
Streptozotocin + Methysergide	38 (n=34)	23 ^d (n=26)	30 (n=27)
Streptozotocin + DSCG	18 (n=22)	10 ^e (n=31)	23 (n=22)
Streptozotocin + Pizotifen	0 ^d (n=32)	13 ^e (n=32)	20 ^e (n=30)
Streptozotocin + Cyproheptadine	30 (n=43)	12 ^e (n=26)	10 ^d (n=29)
Streptozotocin + Prazosin	not determined		

^a Islet sections with ≥ 8 M1/70⁺-cells were regarded as positive for macrophage infiltration; ^b Islet sections with ≥ 4 L3T4⁺- or ≥ 4 Lyt2⁺-cells were counted as positive; *n*=total number of islets. Difference to streptozotocin control, ^c $p < 0.05$; ^d $p < 0.01$; ^e $p < 0.001$

tadine) and all of them largely prevented diabetes development (Fig. 1). Differences in mean blood glucose levels between each of the experimental groups and the streptozotocin control were significant ($p < 0.001$) at day 14 and all later dates.

Treatment with prazosin or with the 4 vasoactive amine antagonists allowed for better growth of animals compared to mice having received streptozotocin alone (data not shown).

Of each experimental group 4–5 animals were killed on day 14 of the experiment and immunocytochemistry of the pancreatic islets was performed. As shown in Table 1, treatment with vasoactive amine antagonists partially prevented infiltration of islets by L3T4⁺-cells. Reduction of infiltration by Lyt2⁺-cells was less pronounced but reached the level of significance for pizotifen and cyproheptadine. Infiltration of pancreatic islets by M1/70.15⁺-macrophages was not affected, except in mice which had received pizotifen.

Discussion

The protective effect of the $\alpha 1$ -adrenoceptor antagonist prazosin shown here suggests that activation of the $\alpha 1$ -adrenoceptor occurs during the pathogenesis of low dose streptozotocin induced diabetes. Treatment with the drug was started only after termination of streptozotocin injections to avoid any direct interaction with the toxin.

It is of interest that administration of the $\alpha 1$ -adrenoceptor antagonist prazosin has been shown previously to suppress increased vascular permeability in experimental autoimmune encephalomyelitis (EAE). Concomitantly clinical signs of the disease were suppressed [7]. In this disease model the beneficial effect of prazosin was explained as drug-mediated inhibition of vasoconstriction with concomitant prevention of va-

soactive amine release and thrombocyte activation. A role for vasoactive amines and thrombocyte products in EAE has also been directly shown by the protection from disease development by treatment with vasoactive amine antagonists [8]. Although an analogy may exist between vascular events in the brain and in islet inflammation, it is not known, at present, whether vasoconstriction occurs during insulitis and whether the protective action of prazosin occurs within the pancreas or at another site.

The contribution of vasoactive amines to the disease process is evident from the inhibition studies described here. Diabetes development was suppressed when animals were treated with either the serotonin antagonists methysergide or cyproheptadine, with pizotifen, an inhibitor of serotonin and histamine action, or with DSCG, an inhibitor of mast cell degranulation.

Enhanced vascular permeability in islets may cause or enhance B-islet cell damage by a number of ways. For one, vasoconstriction and oedema formation is generally accompanied by a state of hypoxia. The extremely low radical scavenger capacity of B cells [9] should render them highly vulnerable to such a condition. Secondly, the lack of an intact endothelial barrier may allow the influx of soluble mediators of the immune system such as toxins, complement components and antibodies. Thirdly, vasoactive amines may promote the infiltration of islets by L3T4⁺- and Lyt2⁺-T-cells. Indeed, we found that the administration of vasoactive amine antagonists largely prevented T-lymphocyte infiltration in pancreatic islets. The effect was more pronounced for L3T4⁺- than for Lyt2⁺-cells. Macrophage infiltration was not affected except for pizotifen. As has been reported [10] serotonin promotes tissue infiltration of T-lymphocytes but not of macrophages. In the low-dose streptozotocin model macrophage infiltration was found independent of T-lymphocyte activity, preceding lymphocyte invasion and occurring also in T-lymphocyte depleted animals [7]. Thus, macrophage invasion of islets seems to be hardly modulated by vasoactive amines. The unexpected complete inhibition of macrophage infiltration of islets in the presence of pizotifen contrasts with the other 3 vasoactive amine antagonists and indicates an additional activity of pizotifen.

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