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INTERLEUKIN-1 RECEPTOR ANTAGONIST PREVENTS LOW DOSE STREPTOZOTOCIN INDUCED DIABETES IN MICE

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The effect of the interleukin-1 receptor antagonist (IL-1ra) on development of hyperglycemia and insulitis in mice treated with multiple low doses of streptozotocin (STZ) was evaluated. C57BL/Ks mice were subjected to the following treatments: 1) Injections i.p. of phosphate-buffered saline (PBS) alone; 2) STZ (5 x 40 mg/kg bw); 3) STZ + PBS delivered by an osmotic pump implanted s.c; 4) STZ + IL-1ra delivered by an osmotic pump (≈ 8 mg/kg bw) for 12-14 days starting on day 5, the last day of STZ injection. IL-1ra had a clear protective action against hyperglycemia and insulitis until day 19, i.e., on cessation of IL-1ra administration. Thus, the results indicate that sustained administration of IL-1ra can prevent the diabetogenic process elicited by low dose STZ treatment, suggesting that IL-1 may have a role in the pathogenesis of this form of diabetes.

Cytokines, in particular interleukin-1 (IL-1), have been postulated as mediators of autoimmune pancreatic β-cell destruction in insulin-dependent diabetes mellitus (IDDM) (1,2). This hypothesis is mostly based on in vitro findings showing inhibitory effects of IL-1 on rodent β-cell preparations (3-5). However, attempts to reduce IL-1 activity in animal models of IDDM are scarce. In this context the naturally occuring IL-1 receptor antagonist (IL-1ra), a competitive inhibitor of IL-1 activity (6), is of special interest. We have previously shown that human IL-1ra can completely block the inhibitory actions induced by IL-1 in rodent β-cells in vitro (7,8). Moreover, in recent experiments we demonstrated that administration of murine IL-1ra delayed pancreatic islet allograft rejection in mice (9). Against this background, we have currently examined the effect of IL-1ra on the incidence of hyperglycemia and insulitis in mice injected with multiple low doses of streptozotocin (STZ; 10).

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Materials and Methods

In vitro experiments

Islet insulin release. Pancreatic islets were isolated from adult Sprague-Dawley rats using a collagenase digestion method and maintained in culture as previously described (11). Groups of 20 islets were then exposed to human IL-1β (1 ng/ml; kindly provided by Dr K. Bendtzen, Copenhagen, Denmark), murine IL-1ra (104 ng/ml; a gift from Dr MJ Bienkowski, Upjohn Co, Kalamazoo, MI) or a mixture of IL-1β and IL-1ra for 2 h. The islets were then washed, cultured overnight and the insulin release determined at 16.7 mM glucose during a 2 h incubation (8).

In vivo experiments

Animals and treatment. Inbred C57BL/Ks male mice (Biomedical Centre, Uppsala, Sweden), originally obtained from the Jackson Laboratory (Bar Harbor, ME) were used. The animals had free access to tap water and pelleted food (R34; AnaLyzen, Lidköping, Sweden). The mice were allocated to four different experimental groups:

1) Intraperitoneal injections of phosphate-buffered saline (PBS) alone for five days.
2) Intraperitoneal injections of STZ (40 mg/kg body weight) for five consecutive days.
3) STZ + PBS delivered by an osmotic pump implanted subcutaneously.
4) STZ + murine IL-1ra (≈ 8 mg/kg body weight/day) delivered by an osmotic pump operating for 12-14 days (9).

In groups 3 and 4 the pumps were implanted on day 5, one hour after the last PBS/STZ injection. For details concerning pump implantation see (9).

Monitored parameters. Glucose determinations (ExacTech blood glucose meter; Baxter Travenol, Deerfield, IL) were performed on blood samples taken from the tail tip on day 1 before any injection, and on days 5, 9, 13, 17 and 19. Some animals were followed further, and their blood glucose levels also measured on days 22, 25 and 29.

Morphology. After killing of the mice, the pancreatic glands were removed, fixed in 10% formalin solution and embedded in paraffin. Sections, 7 μm thick were cut and stained with hematoxylin and eosin. Pancreatic islet histology was ranked according to an arbitrary scale as illustrated previously (12). Score 1 denotes normal islet structure; score 2 denotes mononuclear cell infiltration in the islet periinsular area; score 3 denotes heavy mononuclear cell infiltration into a majority of islets i. e. insulitis; score 4 denotes only a few residual islets present often showing an altered architecture and pyknotic cell nuclei. The pancreatic sections were evaluated by two independent examiners being unaware of the origin of the sections.

Pancreatic insulin concentration. Pieces from different parts of the removed pancreatic glands were cut and homogenized in redistilled water. A fraction of the water homogenate was mixed with acid ethanol and insulin extracted overnight at 4°C before determination of insulin by RIA (13). Another fraction of the aqueous homogenate was used for DNA measurement (14).

Statistical analysis

Values are means ± SEM. When multiple comparisons were performed the data were compared by ANOVA and Fisher's PLSD test, using StatView® (Abacus Concepts, Calabasas, CA). Chi-square test was performed when comparing the number of animals becoming diabetic in the different experimental groups.

Results

In vitro experiments

IL-1β exposure of cultured rat pancreatic islets decreased glucose-stimulated insulin release to 22 ± 5 % of values obtained in the control group, not exposed to either IL-1β

or IL-1ra, ($P<0.001$; n=4). Control islets released 232 ± 35 ng insulin/10 islets x 2 h. When IL-1ra was added together with IL-1β to the culture medium, the insulin secretion was 100 ± 28 % compared to the control group n=4). IL-1ra itself did not affect insulin secretion (data not shown). These results indicate that the murine IL-1ra preparation used countertacted IL-1β elicited actions in vitro on isolated rat islets, and thus closely resemble our previous findings with human IL-1ra (7,8)

In vivo experiments

Two (Group 1 and 2) out of 62 mice died during the course of the study. All PBS treated mice (Group 1; n=15) were normoglycemic (blood glucose level <11.1 mM) throughout the study (Fig. 1). All mice treated with multiple low doses of STZ only (Group 2; n=13) or STZ + PBS delivered via osmotic pump (Group 3; n=16) became gradually hyperglycemic ($P<0.01$ vs PBS alone for both groups; Chi-square test) (Fig. 1). Quite in contrast, 12 out of 16 mice were normoglycemic after treatment with STZ + IL-1ra (Group 4; n=16) on day 19 ($P>0.05$ vs PBS group; Chi-square test). However, from day 19 after implantation of the IL-1ra pump and onwards, i.e.,in conjunction to the cessation of IL-1ra delivery, 7/8 living mice in group 4 presented increasing blood glucose concentrations, and became diabetic between days 19-29 (Fig. 1).

Islet morphological examinations of pancreatic sections of the mice receiving PBS

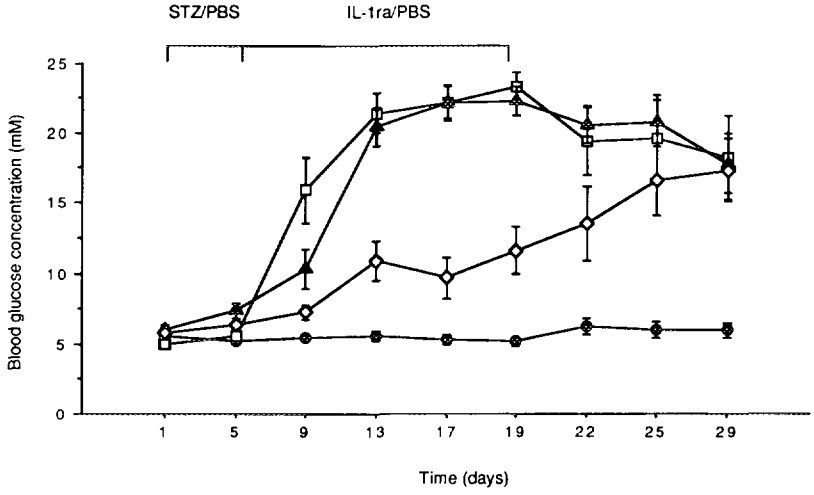

Figure 1. Blood glucose concentrations in C57BL/Ks mice treated with phosphate-buffered saline (PBS) alone (closed circles; n=15); streptozotocin (STZ; 40 mg/kg body weight for 5 consecutive days) alone (open squares; n=13); STZ + PBS (closed triangles; n=16); STZ + IL-1ra (≈ 8 mg/kg body weight/day) (open diamonds; n=16). Values are means ± SEM for n animals. The time interval for the i.p. PBS/STZ and PBS/IL-1ra s.c. osmotic pump treatment is indicated

TABLE 1

Pancreatic islet morphology rank and pancreatic insulin concentration

Treatment	Islet morphology rank		Pancreatic insulin concentration	
	Day 19	Day 29	Day 19	Day 29
			(ng insulin /μg DNA)	
PBS	1.0 ± 0.0	1.0 ± 0.0	13.6 ± 4.4	12.9 ± 3.4
STZ	3.4 ± 0.3***	2.5 ± 0.4***	2.3 ± 1.2***	1.4 ± 0.2***
STZ + PBS	3.0 ± 0.0***	2.9 ± 0.3***	4.1 ± 1.2**	1.4 ± 0.3***
STZ + IL-1ra	2.0 ± 0.3**, ##	2.8 ± 0.3***	6.3 ± 0.3***	1.5 ± 0.3***

The degree of islet insulitis and degeneration was ranked according to an arbitrary scale (1-4), as described in the Materials and Methods section. The pancreatic insulin concentration was measured after homogenisation of pieces of pancreas and extraction in acid ethanol. Insulin was measured by RIA and DNA by fluorophotometry. Values are means ± SEM for 6-8 animals in each group. ** and *** denote $P<0.01$ and $P<0.001$ vs the PBS group on the corresponding day and ## denotes $P<0.01$ vs STZ + PBS group on day 19, using ANOVA and Fisher's PLSD test.

injections only, performed on days 19 and 29, showed a normal islet structure and no cell infiltration (Table 1). All animals treated with STZ injections only or STZ in combination with PBS osmotic pump treatment revealed various stages of mononuclear cell infiltration and/or degeneration of their islets (Table 1). In animals treated with STZ + IL-1ra there was a significantly lower degree of islet mononuclear cell infiltration on day 19. However, on day 29 cellular infiltration was observed in a majority of the islets also in the latter group.

The pancreatic insulin/DNA concentration on day 19 was markedly decreased in the STZ and STZ + PBS groups (Table 1). Also the STZ + IL-1ra group exhibited a decreased pancreatic insulin concentration on day 19, and on day 29 the pancreatic insulin concentration in that group was decreased to the same extent as for the two other STZ groups.

Discussion

The present findings suggest that IL-1ra counteracts islet mononuclear cell infiltration and hyperglycemia, induced by multiple low dose STZ injections in mice. The protective action of IL-1ra seems to be dependent on a sustained administration of the peptide. This is suggested by the fact that the mice gradually developed hyperglycemia after day 19, a time point when the osmotic pumps cease to deliver

IL-1ra (9). In line with this is a similar observation made in mice treated with multiple low dose STZ injections plus the immunosuppressive drug 15-deoxyspergualin (15). Moreover, prevention of mouse islet allograft rejection was achieved only as long as IL-1ra was delivered (9).

The protective action of IL-1ra on day 19 appears to be partial, since 4/16 mice in group 4 were already hyperglycemic and the pancreatic insulin concentration was decreased. This is probably due to that IL-1ra could not protect against the direct β-cell toxicity induced by STZ, but rather IL-1ra affected the anti-islet immune response evoked by the STZ regimen. Note that the IL-1ra treatment was started after the last STZ injection. Indeed, earlier studies have shown in this animal model of IDDM a major contribution of STZ β-cytotoxicity, besides the immune mechanisms, for the development of hyperglycemia (16,17).

From the present study it is not possible to discern to what extent the beneficial action of IL-1ra against IDDM reflects a reduction of direct IL-1 mediated β-cell toxicity or reflects suppression of other systemic immune mechanisms dependent on IL-1 activation. Nevertheless the present data suggest that the cytokine IL-1 might be an interesting target for immune intervention in IDDM. Provided suitable preparations for long-term (3-4 months) in vivo treatment become available, it seems worthwhile to test the efficaccy of IL-1ra on the diabetes incidence in spontaneous animal models of IDDM.

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