Cytotoxic action of IL-1β against pancreatic islets is mediated via nitric oxide formation and is inhibited by $N^G$-monomethyl-L-arginine

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Received 13 January 1992

IL-1β has been previously shown to act as a cytotoxic agent in islets. Here we show by electron microscopy of alginate encapsulated islets, that islet cell lysis is induced by culturing islets for 24 or 48 h in the presence of IL-1β. The extent of lysis depends on the IL-1β concentration and is slightly enhanced by the addition of TNF-α. Cells can be protected from lysis by $N^G$-monomethyl-L-arginine. Lysis is paralleled by an increase in nitrite concentration in culture supernatants of whole islets but not in supernatants of isolated endocrine cells. The results indicate that IL-1β toxicity occurs via inducing in non-endocrine islet cells the synthesis and release of nitric oxide, which has been shown earlier to be highly toxic for islet cells.

Diabetes; Cytotoxicity; Interleukin-1β; Nitric oxide; Arginine analogue; Rat

1. INTRODUCTION

Progressive auto-destruction of pancreatic insulin-producing beta cells leads to type I insulin-dependent diabetes mellitus. Macrophages have been repeatedly shown to play a crucial role in early phases of disease development in animal models: they constitute the major infiltrating cell type during early insulitis in BB rats [1], NOD mice [2] and low-dose streptozotocin-treated mice [3]. Impairment of macrophage function prevents disease manifestation in these animals [3-5]. Activated but not resident macrophages were found to rapidly and effectively kill islet cells but not hepatocytes or thyrocytes [6].

The macrophage product, IL-1β, has been found to cause functional impairment and lysis of islet cells [7]. IL-1β alone or in synergy with TNF-α and IFN-γ was shown to slowly kill islet cells in isolated islets or perfused pancreata [8-10].

We have recently demonstrated that activated macrophages rapidly kill islet cells via arginine-dependent nitric oxide generation [11]. We now provide evidence that IL-1β-mediated cytotoxicity involves nitric oxide formation by subpopulations of cells within the islet.

2. MATERIALS AND METHODS

2.1. Materials
Sodium alginate of low viscosity from Macrocystis pyriformis, $N^G$-monomethyl-L-arginine (NMA) and $N^G$-nitro-L-arginine-methylester (NAME) were from Sigma (Deisenhofen, Germany), rhu IL-1β (>5 × 10^6 U/mg) and TNF-α (2 × 10^7 U/mg) from Genset (Cambridge, USA).

2.2. Animals
Male Lewis rats (200 g) from a local breeding source received a standard diet (‘sniff-R’, Ssniff, Soest, Germany) and tap water ad libitum.

2.3. Encapsulation of islets in alginate beads
Rat pancreatic islets were harvested by ductal injection of collagenase, submitted to gradient centrifugation and cultured in RPMI/10% FCS for 16-18 h for recovery exactly as described previously [12]. Subsequently 7-8 islets of medium size were suspended under the microscope in a degassed alginate stock solution (4% alginate dissolved in water) in 20 mM TRIS-HCl/100 mM NaCl (TBS), pH 7.3). 3 μl of the suspension were carefully gelated in TBS containing 50 mM CaCl₂ and washed with TBS and RPMI/10% FCS.

2.4. Cell culture
Single alginate beads containing the isolated islets were incubated in 400 μl RPMI/10% FCS in 1.5 ml micro sample tubes (Sarstedt, Nümbrecht-Rommelsdorf, Germany) in the absence or presence of cytokines and L-arginine derivatives.

2.5. Electron microscopy
Fixation, dehydration and embedding of the alginate-encapsulated islets were all carried out in the micro sample tubes exactly as described before [12]. From each preparation micrographs of equatorial sections of 5-7 different islets were used to determine the percentage of lysis by measuring lysed vs. intact islet areas. Each experiment was performed 2-3 times.

2.6. Nitrite determination
To measure the amount of nitrite generated during islet culture 30
non-encapsulated islets were cultured in 200 µl RPMI + FCS for 72 h in the presence of the compounds indicated and supernatants removed for measurements of nitrite. Islets were always cultured for 24 h prior to onset of experiments. Nitrite concentrations in culture supernatants were determined by diazotization reaction as described using NaNO₂ as a standard [13].

3. RESULTS

Alginate-encapsulated islets were cultured for up to 48 h in RPMI plus 10% FCS in the presence or absence of IL-1β. Specimens were then processed for transmission electron microscopy. On micrographs of whole islet sections the areas of lysed and intact tissue were measured, the alginate capsule protecting loss from lysed cells. Islet cell lysis was easily recognized by broken plasma membranes always accompanied by loss of electron dense cytoplasmic content as described earlier in detail [6,12]. Fig. 1 shows that islet cell lysis is dependent upon both concentration of IL-1β and time of incubation.

When islets were cultured in the presence of both IL-1β plus the arginine antagonist, NMA (0.5 mM), a marked inhibition of lysis was seen. The protective effect of NMA was found with all concentrations of IL-1β (Fig. 2). The slightly enhanced lysis found in the presence of IL-1β (50 U/ml) plus TNF-α (100 U/ml) was equally well inhibited by NMA. However, neither culturing in the absence of arginine nor addition of NAME protected islets significantly (Fig. 2).

From supernatants of islets cultured in the presence of the agents indicated (Fig. 3) the amount of nitrite was measured as an equivalent for the nitric oxide formed. Culturing in the presence of IL-1β leads to a significant increase of nitrite concentration, which is completely inhibited by the addition of NMA (0.5 mM) and partly...
Electron micrographs show that IL-1β-induced islet cell lysis usually occurs in a chequerboard-like pattern (Fig. 4a) and that islets cultured in the presence of IL-1β plus NMA (Fig. 4b) exhibit normal morphology comparable to untreated controls.

4. DISCUSSION

The results described show that the cytotoxic activity of IL-1β towards pancreatic islets has the characteristics of nitric oxide-mediated cytotoxicity: islet lysis is inhibited by the arginine analogue, NMA, and lysis is paralleled by an increase of the nitrite concentration in the supernatants. These data are in accord with our previous observation, namely that activated macrophages lyse isolated islet cells via the arginine-dependent formation of nitric oxide [11]. It is of interest that non-toxic actions of IL-1β on beta islet cells, i.e. the modulation of insulin secretion after glucose challenge, also have been reported [7,10]. And it was shown very recently that the IL-1β-induced inhibition of insulin secretion is also reversible by adding NMA [14,15]. Islets contain a dense capillary network [16] and endothelial cells have been shown to be inducible for generating large amounts of nitric oxide by cytokines, especially IL-1β in synergy with TNF-α and/or IFN-γ [17–20]. Indeed, we find that the slightly enhanced lysis seen in the presence of both IL-1β and TNF was also completely blocked by NMA. The finding that isolated endocrine cells did not generate measurable levels of nitrite in contrast to whole islets further supports the hypothesis that the non-endocrine cells within an islet,
i.e. endothelial cells, fibroblasts and/or resident macrophages, generate toxic amounts of nitric oxide in the presence of IL-1β.

Islets were not protected by omitting L-arginine from the culture medium; this may be due to either a large enough pool of arginine in islets or due to the ability of endothelial cells to form arginine from citrulline [21]. Work is in progress to characterize inducible nitric oxide generation in isolated islet capillary endothelial cells.

Acknowledgements: Supported by grants from the Deutsche Forschungsgemeinschaft, by the Bundesminister für Jugend, Familie, Frauen und Gesundheit and by the Minister für Wissenschaft und Forschung des Landes Nordrhein-Westfalen. We thank Andrea Schlömer and Ulla Lammersen for technical assistance.

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