For Debate

Type 1 (insulin-dependent) diabetes mellitus and nitric oxide

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Islet cell death during the development of Type 1 (insulin-dependent) diabetes mellitus is at present thought to be mediated in part by oxygen radicals and interleukin 1. Interleukin 1 has been suggested to be toxic to islet cells because of the induction of lethal oxygen radical levels [1]. As a consequence antioxidant therapy is being tested in animal models and similar trials in man are being considered.

We would like to point out that the focus on oxygen radicals as the major toxic principle may be too restricted a view. Results of several recent studies lead us to assume that one important mediator of inflammatory islet cell death is the radical nitric oxide (NO). This inorganic gas has recently attracted considerable interest as a pleiotropic mediator which can be secreted by a large number of cells in the body. It is identical to the endothelium derived relaxing factor (EDRF). It also mediates effects of neurotransmitters, inhibits platelet aggregation and has cytostatic and cytotoxic activities towards tumour cells and parasites [2]. NO is synthesized from L-arginine by NO synthase. Small physiological amounts are produced by a calcium-dependent constitutive isoform, large toxic doses are generated by a calcium-independent inducible isoform [2].

The evidence in favour of a role for NO radicals in islet toxicity is as follows. We incubated pancreatic islet cells with activated macrophages which released large amounts of oxygen radicals, interleukin 1, tumour necrosis factor alpha as well as NO radicals. Death of islet cells could not be prevented by oxygen radical scavengers or by neutralisation of interleukin 1 and tumour necrosis factor alpha but only after inhibition of NO synthesis [3]. Whether NO is toxic to islet cells in the absence of any other macrophage product was tested by using a chemical source of NO. When islet cells were exposed to the NO donor nitroprusside, lysis of cells occurred in a concentration- and time-dependent manner [4].

Furthermore, the action of interleukin 1 appears to be linked to the release of NO. We recently observed that interleukin-1-induced islet cell lysis is completely abolished when NO synthesis has been prevented [5]. We therefore assume that interleukin 1 induces massive NO secretion

from intra-islet macrophages which then causes islet cell lysis. This assumption is supported by our observation that under comparable culture conditions interleukin 1 induces substantial amounts of NO and cell lysis with whole islets as targets [5] but not with reaggregated islet cells (pseudoislets) devoid of endothelial cells and macrophages (unpublished data). It has been shown by others that inflammatory cytokines induce the production of high doses of NO by endothelial cells [6]. Thus, in addition to macrophages the endothelium may be an additional source of NO during islet inflammation.

Under non-cytolytic conditions interleukin 1 is known to modulate the insulin response of isolated islets to glucose. Three different laboratories have shown that the suppression of insulin production by interleukin 1 is dependent of NO formation [7–9] while the early increase in insulin release appears to be NO-independent [10]. It has been suggested by Schmidt et al. [11] that the physiological insulin response to L-arginine or tolbutamide involves NO synthase activity. Indeed, the constitutive isoform of this enzyme (producing low, non-toxic NO levels) could be isolated from a Beta-cell line [11]. Thus, at lower concentrations a regulatory role of NO appears to exist, while high doses of NO are cytotoxic to islet cells.

First attempts to suppress Type 1 diabetes development in animal models by inhibiting NO production have been encouraging. Treatment of mice with inhibitors of NO synthase significantly attenuated hyperglycaemia in the low-dose streptozotocin model [12, 13].

These data suggest that NO may be a major mediator of inflammatory islet cell death. This should be considered in strategies of anti-inflammatory therapy for the prevention of Type 1 diabetes. It is of interest that nicotinamide which prevents diabetes in the NOD mouse model [14] and therefore will be evaluated in several multicentre trials does protect islet cells from macrophage-mediated toxicity [15], now known to be primarily NO-mediated [3].

NO interacts with divalent iron of haeme groups and inhibits enzymes with FeS centres [2]. Which of these interactions, or what other effects of NO are responsible for islet cell lysis remains to be determined in further studies.

References

1. Nerup J, Mandrup-Poulson T, Mølvig J, Helqvist S, Wogensén L, Egeberg J (1988) Mechanism of pancreatic β -cell destruction in type I diabetes. *Diabetes Care* 11: 16–23
2. Moncada S, Palmer RMJ, Higgs EA (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacological Rev* 43: 109–142
3. Kröncke KD, Kolb-Bachofen V, Berschick B, Burkart V, Kolb H (1991) Activated macrophages kill pancreatic syngeneic islet cells via arginine-dependent nitric oxide generation. *Biochem Biophys Res Commun* 175: 752–758
4. Burkart V, Kröncke KD, Brenner HH, Kolb H, Kolb-Bachofen V (1991) Nitric oxide is a pathogenetic factor in type 1 diabetes. *Diabetologia* 34 [Suppl 2]: A 95 (Abstract)
5. Bergmann L, Kröncke KD, Suschek C, Kolb H, Kolb-Bachofen V (1992) Cytotoxic action of IL-1 β against pancreatic islets is mediated via nitric oxide formation and is inhibited by N^G-monomethyl-L-arginine. *FEBS Lett* 299: 103–106
6. Radomski MW, Palmer RMJ, Moncada S (1990) Glucocorticoids inhibit the expression of an inducible, but not the constitutive nitric oxide synthase in vascular endothelial cells. *Proc Natl Acad Sci USA* 87: 10043–10047
7. Southern C, Schulster D, Green IC (1990) Inhibition of insulin secretion by interleukin-1 β and tumour necrosis factor- α via an L-arginine-dependent nitric oxide generating mechanism. *FEBS Lett* 276: 42–44
8. Corbett JA, Lancaster JR, Sweetland MA, McDaniel ML (1991) Interleukin-1 β -induced formation of EPR-detectable iron-nitrosyl-complexes in islets of Langerhans. *J Biol Chem* 266: 21351–21354
9. Welsh N, Sandler S (1992) Interleukin-1 β induces nitric oxide production and inhibits the activity of aconitase without decreasing glucose oxidation rates in isolated mouse pancreatic islets. *Biochem Biophys Res Commun* 182: 333–340
10. Welsh N, Eizirik DL, Bendtzen K, Sandler S (1991) Interleukin-1 β -induced nitric oxide production in isolated rat pancreatic islet requires gene transcription and may lead to inhibition of the Krebs cycle enzyme aconitase. *Endocrinology* 129: 3167–3173
11. Schmidt HHHW, Warner TD, Ishii K, Sheng H, Murad F (1991) Insulin secretion from pancreatic β cells caused by L-arginine-derived nitrogen oxides. *Science* 255: 721–723
12. Lukic ML, Stosic-Grujicic S, Ostojic N, Chan WL, Liew FY (1991) Inhibition of nitric oxide generation affects the induction of diabetes by streptozotocin in mice. *Biochem Biophys Res Commun* 178: 913–920
13. Kolb H, Kiesel U, Kröncke KD, Kolb-Bachofen V (1991) Suppression of low dose streptozotocin induced diabetes in mice by administration of nitric oxide synthase inhibitor. *Life Sci* 49: PL 213–PL 217
14. Yamada K, Nonaka K, Hanafusa T et al. (1982) Preventive and therapeutic effects of large-dose nicotinamide injections on diabetes associated with insulinitis. *Diabetes* 31: 749–753
15. Kolb H, Burkart V, Appels B et al. (1990) Essential contribution of macrophages to islet cell destruction in vivo and in vitro. *J Autoimmunity* 3 [Suppl]: 117–120

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Announcement

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