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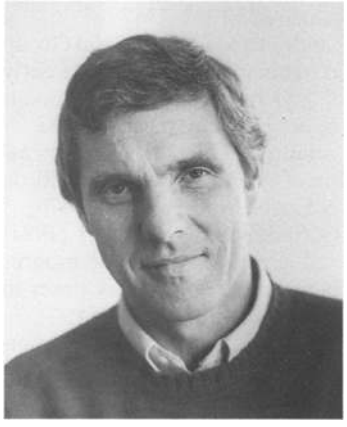
Jørn Nerup
Minkowski Award, 1978, Zagreb



Dr. Nerup obtained his M.D. from the University of Copenhagen in 1964. He completed postgraduate education in Copenhagen, Denmark, the Karolinska Hospital, Stockholm, Sweden, and the Royal Hammersmith Hospital, London, UK. Dr. Nerup obtained specialist degrees in internal medicine (1976) and endocrinology (1984). Since 1976 Dr. Nerup has been Chief Physician at the Steno Diabetes Center, Gentofte Denmark. Between 1989 and 1990 he was Director of the Hagedorn Research Laboratory. Dr. Nerup is Director of the WHO Collaborating Center, Steno Diabetes Center/Hagedorn Research Laboratory for Training in Diabetes Research. Dr. Nerup is currently Professor at the University of Copenhagen. Dr. Nerup has more than 300 publications in the fields of endocrinology, diabetes and immunology. In 1992 Dr. Nerup was chairman of the WHO Study Group on the Prevention of diabetes mellitus.

On the pathogenesis of IDDM

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Summary A model of the pathogenesis of insulin-dependent diabetes mellitus, i.e. the initial phase of beta-cell destruction, is proposed: in a cascade-like fashion efficient antigen presentation, unbalanced cytokine, secretion and poor beta-cell defence result in beta-cell destruction by toxic free radicals (O_2^- and nitric oxide) produced by the beta cells themselves. This entire process is under polygenetic control. [Diabetologia (1994) 37 [Suppl 2]: S 82–S 89]

Key words Insulin-dependent diabetes mellitus, free radicals, cytokines, beta-cell destruction.

Since the first experimental demonstration in 1971 of autoimmunity in IDDM [1] and of the HLA Class I-IDDM association in 1974 [2] and a year later of the HLA Class II-IDDM association [3] more than 5200 papers on immunology and genetics of IDDM in spontaneous animal models and in man have appeared in the literature.

In the 1974 [2] paper the first comprehensive hypothesis concerning the aetiology and pathogenesis of IDDM was presented: "One or more immune-response genes associated with HL-A8 and/or W15 might be responsible for an altered T-lymphocyte response. The genetically determined host response could fail to eliminate an infecting virus (coxsackie virus B4 and others) which in turn might destroy the pancreatic beta cells or trigger an autoimmune reac-

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Abbreviations: IDDM, Insulin-dependent diabetes mellitus; TNF, tumour necrosis factor; IL-1, interleukin-1; BB, biobreeding; NOD, non-obese diabetic; IFN, interferon; APC, antigen presenting cells; mn SOD, manganosuperoxide dismutase; NO, nitric oxide; NOS, nitric oxide synthases; $ONOO^-$, peroxynitrite.

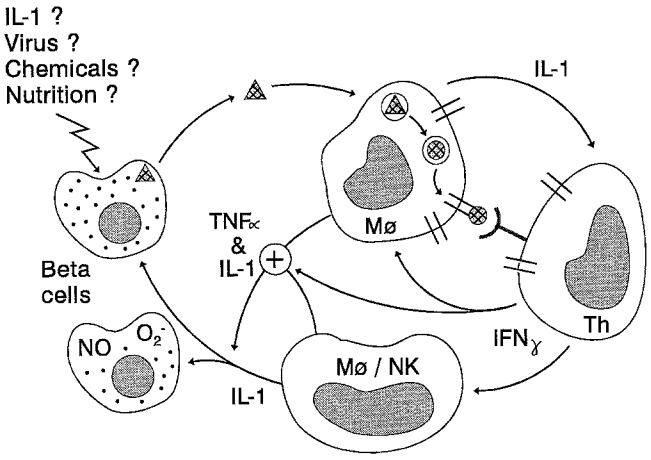

Fig. 1. Pathogenetic model of IDDM

Table 1. Candidate antigens in IDDM

- Insulin/proinsulin
- 38k insulin secretory granule membrane protein
- Carboxypeptidase H
- p 52 rat insulinoma cell membrane protein
- 155 kD rat insulinoma cell membrane protein
- Gangliosides
- GAD 65 kD and 67 kD
- 37/40 kD tryptic fragment of 64 kD non-GAD islet protein
- 32, 55–72, 120–170 kD islet proteins
- GLUT 2
- Peripherin (58 kD)
- Heat shock protein (HSP) 62 and 65
- Bovine serum albumin (BSA)
- ABBOS (linear BSA epitope)
- p 69 BSA homologous peptide
- Sulphatides

tion against the infected organ". On the basis of the flow of new information this hypothesis in 1981 was modified to:

"Genetic predisposition (susceptibility) to IDDM is conferred by two genes on chromosome 6, one associated with HLA-D/DR3, one with HLA-D/DR4. The reaction of susceptible individuals to certain environmental stimuli (beta-cell cytotoxic virus? beta-cell cytotoxic chemicals?) is abnormal, leading to: beta-cell destruction directly, through "autoimmune mechanisms" or because of lack of regeneration of the beta cell after damage."

This *hypothesis* rapidly, however rather uncritically, gained wide acceptance, and it has almost become *dogma* that IDDM is a classic organ-specific autoimmune disease, in which beta cells are destroyed by T-lymphocyte mediated mechanisms and circulating autoantibodies are markers of the ongoing disease process. Evidence to support the participation of classic immune mechanisms in beta-cell destruction is plentiful [4–6]. However, if the term *pathogenesis* defines the earliest events and mechanisms involved in beta-cell destruction, there is ample evidence to support, in spontaneous animal models of IDDM and probably in pre-diabetic man, that a series of distinct events take place well before the invasion of the islet by T lymphocytes and beta-cell destruction by cytotoxic T lymphocytes is possible [7–10]. In other words: beta-cell destruction, hence the pathogenesis of IDDM, comprises two phases. i) a non-lymphocyte-dependent initial phase and ii) a T-lymphocyte mediated amplification and perpetuation phase of beta-cell destruction.

The initial phase of beta-cell destruction: the pathogenesis of IDDM

Based upon our findings, that supernatants of stimulated, non-diabetic human peripheral blood mononuclear cells were cytotoxic to rat and human beta cells in vitro [11, 12], the identification of IL-1 – potentiated by TNF alpha – as the cytotoxic principle [13, 14] and reviews of the literature [6, 15], we proposed a model of the pathogenesis of IDDM (Fig. 1) – the Copenhagen model.

This model gives a pivotal role to the macrophage as an antigen presentor, an accellerator of the immune response and as the producer of the beta-cell cytotoxic molecules, i. e. cytokine secretion.

Little, if any, evidence contradict the model. In fact, several groups confirmed the beta-cell cytotoxicity of cytokines [16–18]. Sequential, morphological studies of the cellular infiltrate of the islets of Langerhans in the BB rat [7, 19] and the NOD mouse [8] demonstrated that cells of the monocyte macrophage dendritic (MO/MØ/DC) lineage are the first on stage, and such cells in situ have been shown to contain abundant mRNA for IL-1 and TNF [20, 21].

Ablation experiments with silica [22, 23] showed that functional macrophages are obligatory for the disease process, whereas passive transfer experiments of spleen cells and specifically reactive T-cell clones to young NOD mice and young NOD/Scid mice suggested, that CD8+ cytotoxic T-lymphocytes are not necessary and CD4+ lymphocytes not sufficient to produce IDDM in the recipients [24].

Furthermore, the very long list of putative beta-cell specific and non-specific "autoantigens" (Table 1) identified by the presence of "autoantibodies" in IDDM sera, is difficult to reconcile with a primary and specific, T-lymphocyte-dependent antigen-driven beta-cell destructive insult.

To us this suggests that beta cells are not destroyed because islet proteins are antigens in their native form. Rather, these proteins become antigens when and because beta cells are destroyed. This means that "autoantibodies" are secondary in importance and time in the pathogenetic process and that the islet proteins are probably made antigenic through a common mechanism.

Table 2. Some important islet cell parameters affected by interleukin 1 in vitro

Biphasic dose/time-dependent effects on:
insulin release; synergy with TNF-alpha
insulin content
glucagon release and content
proinsulin biosynthesis
preproinsulin mRNA
total protein biosynthesis
thymidine incorporation
glucose oxidation; synergy with TNF-alpha (only rat islets)
late Ca^{2+} influx
phosphoinositide metabolism
Decreases in:
aconitase activity
ATP content
oxygen consumption rate
Increases in:
intracellular free sodium
prostaglandin E_2
heat shock protein synthesis
c-fos protooncogene expression
MnSOD activity
NO synthesis
Selective beta-cell lysis

Thus, by definition, this mechanism – beta-cell destruction induced by IL-1 potentiated by TNF, IFN and possibly other cytokines – is in operation prior to the involvement of the "classic" immune system.

We hypothesize that understanding the molecular details of IL-1-induced beta-cell destruction equals understanding the pathogenesis of IDDM and may provide the basis for rational intervention modalities and hopefully the prevention of IDDM.

The model

The model (Fig. 1) predicts that anything from the external or internal environment which can destroy a beta cell (nutrients, virus, chemicals, IL-1) will lead to the release of beta-cell *proteins*. These proteins will be taken up by residing *antigen presenting* cells (MØ, MO, DC) in the islets, will be processed to antigenic peptides and as such be presented by MHC-class II molecules on the cell surface.

This activates the antigen presenting cells to produce and secrete monokines (IL-1, TNF) and co-stimulatory signal(s) which if T-helper lymphocytes with receptors specifically recognizing the antigenic peptide are present in the islet, induce the transcription of a series of lymphokine genes.

One of these, IFN, will feedback-stimulate the antigen presenting cells to increase expression of MHC-class II molecules and IL-1 and TNF. In addition, other cells of the MØ/MO/DC lineage present in the islet are also induced to secrete monokines.

IL-1, potentiated by TNF and IFN is cytotoxic to beta cells through the induction of free radical formation in the islet (see further).

As part of the beta-cell destructive mechanism some beta-cell proteins are damaged ("denatured") by free radicals and in more antigenic forms (linear epitopes) presented to the immune system, thereby closing the loop in a self-perpetuating and self-limiting fashion.

The magnitude of beta-cell destruction will depend upon a) the velocity of the feed-back circuit between the antigen presenting cells and the T-helper lymphocyte, i. e. on the efficacy of antigen presentation/recognition, b) the magnitude of cytokine production, and c) on the capacity of beta-cell defense mechanisms during cytokine exposure.

Each specific element of the pathogenetic process is under genetic control, i. e. IDDM is a polygenic disorder (see further).

The model has important implications, since it eliminates the need for the existence of specific environmental trigger(s), IDDM-specific genes or gene mutations, and targetted exposure of beta cells to the cytotoxic action of monokines.

Further, it offers an explanation for the existing multitude of "autoantibodies" in newly-diagnosed and pre-diabetic IDDM: proteins released during beta-cell destruction by free radicals, may not be released in their native confirmation, but rather in free radical modified ("denatured") more antigenic forms.

Against this background we postulate that the remarkable specificity of cellular destruction in the islets in IDDM is due to an inherent vulnerability of beta cells to cytokine-induced free radical damage. In other words: the beta cells die because they are beta cells.

IL-1 effects on islets in vitro and in vivo: Exposure of rat islets in vitro to IL-1 (recombinant human IL-1β 150 pg/ml, specific activity ~ 400 U/ng, in RPMI 1640 for 24 h) has dramatic effects on beta-cell function and islet morphology (Table 2, Fig. 2).

Longer-term exposure (5–7 days) completely disintegrates islets and destroys beta cells as well as alpha cells. The shorter term exposure does not affect alpha-cell function or morphology, but induces severe inhibition of insulin release and heavy damage to beta cells ranging from extensive degranulation to complete cytolysis [25].

Repeated exposure alternating with 5-day recovery periods in IL-1 free medium, has increasingly devastating effects on insulin secretion and its recovery. Glucagon secretion, however, is unaffected by repeated IL-1 exposure [26]. These in vitro findings were confirmed in experiments on the intact pancreatic gland in situ [27] and a diabetes-like condition characterized by hyperglycaemia and hypoinsulin-

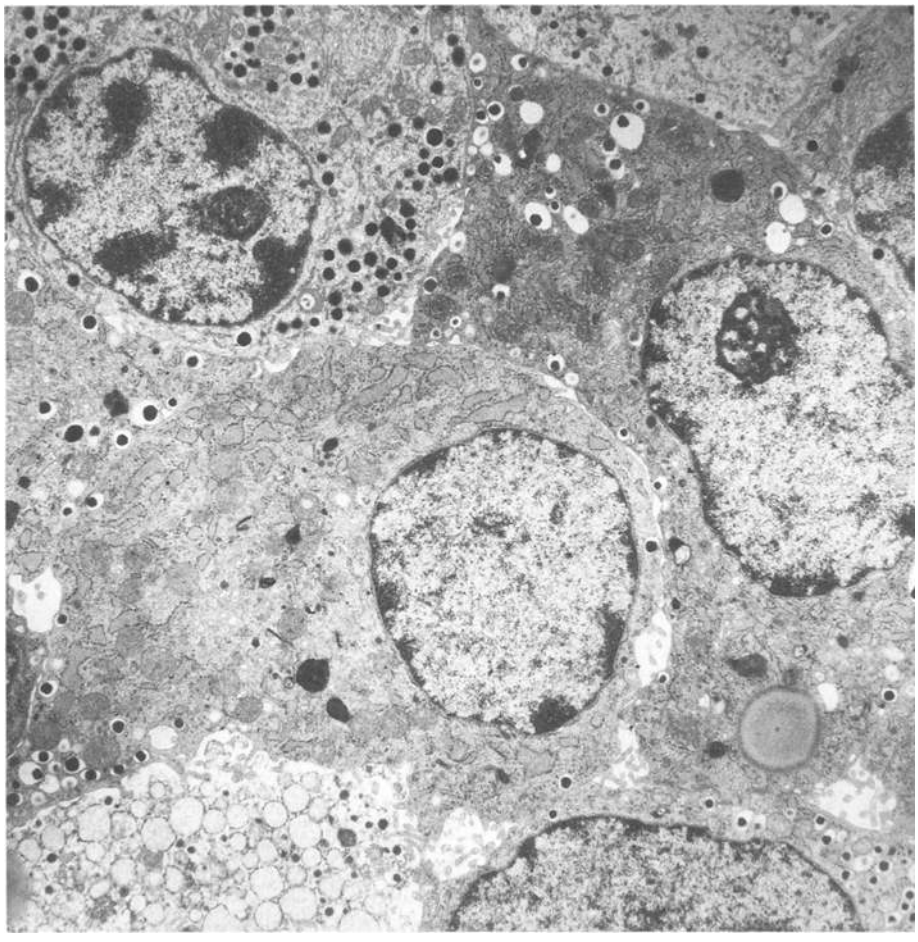

Fig. 2. Electron microscopy photograph (× 5000) of a rat islet of Langerhans showing severe beta-cell damage (lower left) and moderate beta-cell damage (right) and intact alpha cell (upper left)

aemia is seen in normal rats receiving daily IL-1 injections (4 μg per kg body weight) i.p. for 5 days [28]. Mouse islets in culture are more resistant to IL-1 cytotoxicity than rat islets. By increasing the low calcium concentration (0.42 mmol/l) of RPMI 1640 to near physiological concentrations, the IL-1 sensitivity of mouse islets becomes similar to that of rat islets [29]. Few studies of IL-1 effects on human islets in culture have been reported and with conflicting results. Almost complete resistance [30] and "normal" sensitivity i.e. similar to the islets, [31] have been found. More studies are required to solve this controversy and to reveal the reasons behind strain [32], species and possibly individual differences in beta-cell sensitivity to IL-1 cytotoxicity.

IL-1 induced beta-cell cytotoxicity in vitro is dependent upon energy generation, DNA synthesis and metabolic activity of the beta cell [33, 34]. This led us to suggest that the beta cell may be seen as a "moving target". The "resting" beta cell is more resistant than the "active" beta cell, e.g. low glucose, amiloride and somatostatin shift the dose-response curve to the right; high glucose and leucine in the culture medium to the left [33–35].

The molecular mediators of IL-1 induced beta-cell destruction

The IL-1 induced phenomena listed in Table 2 all take hours to occur. Searching for a common, rapidly acting, mechanism capable of explaining the selectivity of beta-cell destruction as well as the diverse phenomena in Table 2, we suggested a role for free radicals first in the form of superoxide anions O_2^- [36]. This concept was supported by a series of observations. In brief: IL-1 activates the Na^+/H^+ (antiport) system [35], which may lead to O_2^- formation in the mitochondria as seen in other tissues [37].

IL-1 induces an IL-1 and islet specific oxidative stress protein (alternative defense against O_2^-) response in islets in vitro [38]. An identical response could be induced by islet exposure to H_2O_2 [38]. Beta-cell free radical scavenging potential is low, due to low content of MnSOD [39]. O_2^- scavenging molecules protect beta cells against cytokine induced cytotoxicity [40].

Accordingly, beta cells are not exposed to specific cytotoxic mechanisms. IL-1 (and TNF) may also in non-beta cells induce formation of O_2^- as well, the effects of which are neutralized by activation and increased expression of scavenger enzymes (e.g.

MnSOD) for which IL-1, potentiated by TNF, is the natural inducer. Thus, in all IL-1 receptor-bearing cell types, IL-1 initiates a kind of race between protective and deleterious events. In beta cells the deleterious prevail. Consequently, we hypothesize that beta cells are exquisitely IL-1 sensitive because of their low content and inducibility of the scavengers.

Our recent in situ hybridization experiments support this by showing that only moderate (less than two-fold) increases in expression levels of MnSOD and haeme oxygenases 1 and 2 are induced by IL-1 in rat islets during 24- to 48-h incubation periods (Cuartero, unpublished observation).

The observation by Southern et al. [41] that islets exposed to IL-1 release nitrite as an indication of the induction of another free radical species NO, and the demonstration [42], that NO produced chemically is cytotoxic to islet cells further substantiates a role for free radicals in beta-cell destruction.

NO is produced during the conversion of L-arginine to citrulline by a family of enzymes, the nitric oxide synthases (NOS). Three isoforms of NOS exist, two constitutive forms (cNOS) cloned from brain and endothelial cells (both Ca^{2+} dependent, produced in small amounts for short periods of time) and one inducible form (iNOS) which is Ca^{2+} independent and produced in large amounts for longer periods of time in macrophages, hepatocytes, smooth muscle cells and endothelial cells [43].

cNOS and iNOS have about 65 % sequence homology. The sequences of the iNOSs are identical within and almost identical between species (mouse/rat). Although structurally identical the iNOSs require different inducers for their expression, e.g. endotoxin and IFN on macrophages [44], and IL-1 on chondrocytes and smooth muscle cells [45, 46]. This suggests, that inducer-specific signal transduction pathways and/or DNA regulator sequences exist in the genes in different iNOS producing cell types. Rat islets cultured in RPMI 1640 show no spontaneous iNOS synthesis as evidenced by mRNA and nitrite release (< 20 pmol). However, when exposed to IL-1, mRNA is detectable after 30–60 min. (Cuartero, unpublished observation) and nitrite release is seen after 8–10 h, continuing for 24 h or more to impressive levels (approximately 10 pmol · islet^{-1} · 24 h^{-1}) [41, 47].

From IL-1 stimulated rat islets we have cloned an iNOS with a coding sequence nearly identical to that of the other rat iNOSs [48]. This iNOS can be induced by IL-1 in RIN-cells and by a mixture of cytokines (IL-1, TNF, IFN) in human islets [47] as measured by nitrite release to the tissue culture medium. The cloning and sequencing of the human islet iNOS is in progress. The iNOS gene has been mapped to mouse chromosome 11 (syntenic regions on chromosomes 10 and 17 in rat and man respectively) [49].

It has been a matter of debate in which cells in the islets NO is produced. The Düsseldorf group [50] maintains that NO is released by macrophages and endothelial cells, whereas we and the St. Louis group [51] see the beta cell itself as the major site of production because: 1) in islets exposed in vitro to NO produced by chemical NO donors both alpha- and beta-cells are destroyed [52]. 2) Human macrophages are still to be shown capable of producing NO. 3) IL-1 induces iNOS synthesis in sorted beta cells, but not in alpha cells [53].

However, in the intact islet of Langerhans in vivo during the disease process, most likely beta cells and endothelial cells as well as macrophages are involved as NO producers and thereby in beta-cell destruction.

The involvement of iNOS synthesis in beta-cell destruction explains the need for energy generation and protein synthesis during the process of beta-cell destruction. In fact, more than 25 islet proteins are upregulated during IL-1 exposure [47]. The finding that increases in glucose concentrations lead to upregulation of iNOS expression (Andersen, unpublished observation), supports the moving target concept. Little is known, however, about the regulation of iNOS gene expression and such studies are clearly in demand. iNOS expression has also been demonstrated in islets of diabetes prone BB-rats close to the onset of diabetes [54].

Although a major player, NO formation alone cannot fully explain the devastating effects of IL-1 exposure on beta cells. Rat islets cultured in arginine-free RPMI 1640 produce no NO when stimulated with IL-1. Nevertheless, the initial increase in insulin release is preserved, and the later decrease of insulin release is inhibited by only about 50 % [41, 55], supporting the existence of an NO-independent (O_2^- dependent?) pathway of IL-1 effects on beta-cell function and survival.

The molecular mechanisms of IL-1 induced, free radical mediated beta-cell destruction

Beta cells do express IL-1 receptors [31, 56], but very little is known about receptor signalling pathways in any IL-1 responsive cells. Hence, how IL-1 exposure alone (or in combination with TNF and IFN) leads to alterations in gene-expression, notably of insulin and iNOS, is not known at present. How the mediators of IL-1 induced beta-cell destruction, the free radicals O_2^- and NO^-, may be generated, we have attempted to describe above. The consequences of their occurrence are shown in a very simplified schematic form in Figure 3.

Free radicals are known to produce DNA strand breaks [41, 57] to be followed by poly(ADP)ribose polymerase activation and NAD depletion resulting in cell death. NO has been demonstrated by electron

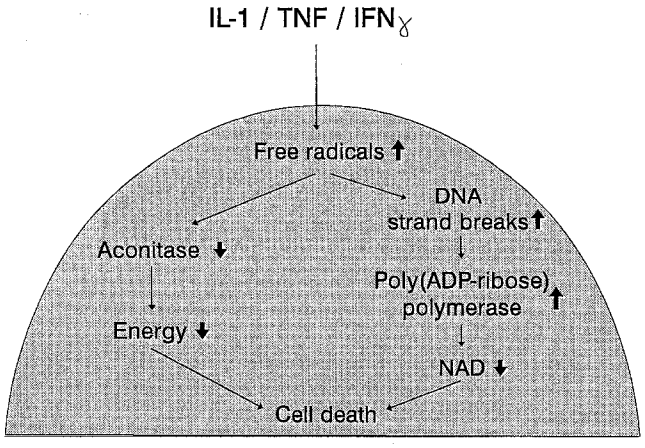

Fig. 3. Solematic model of cytokine-induced molecular mechanisms of beta-cell destruction

paramagnetic resonance to form iron-nitrosyl complexes [58]. This will inactivate important enzymes, e.g. aconitase, which IL-1 has been shown to do [59]. The consequence is decreased ATP generation and subsequent cell death.

In addition NO and O_2^- may modify other protein functions by nitrosylation and oxidation.

When not scavenged, due to low beta-cell scavenging potential, O_2^- may react with NO to form the powerful oxidant peroxynitrite ($ONOO^-$) a source of the very toxic hydroxyl (OH^-) free radical [60].

$ONOO^-$, and probably also NO and O_2^- may "denature" beta-cell proteins. Finally, $ONOO^-$ may react with metalloproteins, e.g. CuZnSOD [61], to form a nitronium-like intermediate, which nitrates tyrosine residues on intracellular signalling molecules such as tyrosine kinases.

This should provide mechanisms enough to explain beta-cell destruction during IL-1 exposure. It may further explain how beta-cell proteins become beta-cell antigens through a common mechanism because and when beta cells are destroyed. We suggest, that this is what triggers the T-lymphocyte dependent amplification and perpertuation phase of the pathogenetic process leading to IDDM.

The process of cytokine-induced beta-cell destruction is, however, probably rather more complicated than suggested in this paper. IL-1 induces the upregulation of more than 25 islet proteins as demonstrated by 2D-SDS gel studies [47]. When islets are protected against IL-1 cytotoxicity, e.g. by nicotinamide [55], arginine free culture conditions [41, 55] or by L-arginine analogues the number of protein changes is considerably reduced.

The pathogenetic model (Fig. 1) implies that IDDM in man is a highly polygenic disorder. The polygenic nature of IDDM is supported by findings in the NOD mouse [62] and the BB-rat [63]. We have proposed [reviewed in 10], that in human IDDM common alleles of normal genes recurring in unfavourable combinations confer susceptibility to IDDM by encoding a complex phenotype characterized by i) efficient antigen presentation (HLA-class II), ii) unbalanced cytokine secretion and iii) poor beta-cell defence mechanisms.

We expect that the identification of these genes, their corresponding proteins and their functions will finally lead to the full understanding of beta-cell destruction at the molecular level and in the end rational approaches to the prevention and cure of IDDM.

References

1. Nerup J, Andersen OO, Bendixen G, Egeberg J, Poulsen JE (1971) Antipancreatic cellular hypersensitivity in diabetes mellitus. Diabetes 20: 424–427
2. Nerup J, Platz P, Andersen OO et al. (1974) HL-A antigens and diabetes mellitus. Lancet I: 864–866
3. Thomsen M, Platz P, Andersen OO et al. (1975) MLC typing in juvenile diabetes mellitus and ideopathic Addisons disease. Transpl Rev 22: 125–147
4. Eisenbarth GS (1986) Type 1 diabetes mellitus. A chronic autoimmune disease. N Engl J Med 314: 1360–1368
5. Mandrup-Poulsen T, Helqvist S, Mølvig J, Wogensen LD, Nerup J (1989) Cytokines as immune effector molecules in autoimmune endocrine diseases with special reference to insulin-dependent diabetes mellitus. Autoimmunity 4: 191–218
6. Nerup J, Mandrup-Poulsen T, Mølvig J, Helqvist S, Wogensen LD (1989) On the pathogenesis of insulin-dependent diabetes mellitus – a discussion of three recently proposed models. In: Creutzfeldt W, Lefèbvre P (eds) Diabetes mellitus: pathophysiology and therapy. Springer-Verlag, Berlin, pp 39–50
7. Hanenberg H, Kolb-Bachofen V, Kantwerk-Funke G, Kolb H (1989) Macrophage infiltration precedes and is prerequisite for lymphocytic insulitis in pancreatic islets of pre-diabetic BB rats. Diabetologia 32: 126–134
8. O'Reilly LA, Hutchings PR, Crocker PR et al. (1991) Characterization of pancreatic islet cell infiltrates in NOD mice: effect of cell transfer and transgene expression. Eur J Immunol 21: 1171–1180
9. Mandrup-Poulsen T (1988) On the pathogenesis of insulin-dependent diabetes mellitus. Dan Med Bull 35: 438–460
10. Mølvig J (1992) A model of the pathogenesis of insulin-dependent diabetes mellitus. Dan Med Bull 39: 509–541
11. Mandrup-Poulsen T, Bendtzen K, Høiriis Nielsen J, Bendixen G, Nerup J (1985) Cytokines cause structural and functional damage to isolated islets of Langerhans. Allergy 40: 424–429
12. Mandrup-Poulsen T, Bendtzen K, Nerup J, Egeberg J, Nielsen JH (1985) Mechanism of pancreatic islet cell destruction. Dose-dependent cytotoxic effect of soluble blood mononuclear cell mediators on isolated islets of Langerhans. Allergy 41: 250–259
13. Mandrup-Poulsen T, Bendtzen K, Nerup J, Dinarello CA, Svenson M, Nielsen JH (1986) Affinity purified human Interleukin 1 is cytotoxic to isolated islets of Langerhans. Diabetologia 29: 63–67
14. Mandrup-Poulsen T, Bendtzen K, Dinarello CA, Nerup J (1987) Human tumor necrosis factor potentiates human interleukin 1-mediated rat pancreatic β-cell cytotoxicity. J Immunol 39: 4077–4082

15. Nerup J, Mandrup-Poulsen T, Mølvig J, Helqvist S, Wogensen L, Egeberg J (1988) Mechanisms of pancreatic β-cell destruction in type 1 diabetes. Diabetes Care 11 [Suppl 1]: 16–23
16. Sandler S, Eizirik DL, Svensson C, Strandell E, Welsh M, Welsh N (1991) Biochemical and molecular actions of interleukin-1 on pancreatic β-cells. Autoimmunity 10: 241–253
17. Rabinovitch A, Pukel C, Baquerizo H (1988) Interleukin-1 inhibits glucose-modulated insulin and glucagon secretion in rat islet monolayer cultures. Endocrinol 122: 2393–2398
18. McDaniel ML, Hughes JH, Wolf BA, Easom RA, Turk JW (1988) Descriptive and mechanistic considerations of interleukin 1 and insulin secretion. Diabetes 37: 1311–1315
19. Voorbij HAM, Jeucken PHM, Kabel PJ, de Haan M, Drexhage HA (1989) Dendritic cells and scavenger macrophages in pancreatic islets of prediabetic BB rats. Diabetes 38: 1623–1629
20. Jiang Z, Woda BA (1991) Cytokine gene expression in the islets of the diabetic Biobreeding/Worcester rat. J Immunol 146: 2990–2994
21. Held W, MacDonald HR, Weissman IL, Hess MW, Mueller C (1990) Genes encoding tumor necrosis factor α and granzyme A are expressed during development of autoimmune diabetes. Proc Natl Acad Sci USA 87: 2239–2243
22. Oschilewski U, Kiesel U, Kolb H (1985) Administration of silica prevents diabetes in BB-rats. Diabetes 34: 197–199
23. Lee KU, Amano K, Yoon JW (1988) Evidence for initial involvement of macrophage in development of insulitis in NOD mice. Diabetes 37: 989–991
24. Haskins K, McDuffie M (1990) Acceleration of diabetes in young NOD mice with a CD4+ islet-specific T-cell clone. Science 249: 1433–1436
25. Mandrup-Poulsen T, Egeberg J, Nerup J, Bendtzen K, Nielsen JH, Dinarello CA (1987) Ultra-structural studies of time-course and cellular specificity of interleukin 1 mediated islet cytotoxicity. Acta Path Microbiol Immunol Scand (C) 95: 55–63
26. Helqvist S, Zumsteg UW, Spinas GA et al. (1991) Repetitive exposure of pancreatic islets to interleukin-1β. An in vitro model of prediabetes? Autoimmunity 10: 311–318
27. Wogensen LD, Kolb-Bachofen V, Christensen P (1990) Functional and morphological effects of interleukin-1β on the perfused rat pancreas. Diabetologia 33: 15–23
28. Wogensen L, Reimers J, Nerup J (1992) Repetitive in vivo treatment with human recombinant Interleukin 1β modify beta-cell function in normal rats. Diabetologia 35: 331–339
29. Helqvist S, Bouchelouche P, Andersen HU, Nerup J (1989) Modulation of calciumflux influences interleukin 1β effects on insulin release from isolated islets of Langerhans. Acta Endocrinol (Copenh) 121: 447–455
30. Eizirik DL, Welsh N, Hellerström C (1993) Predominance of stimulatory effects of interleukin-1β on isolated human pancreatic islets. J Clin Endo Metab 76: 399–403
31. Zumsteg U, Reimers JI, Pociot F et al. (1993) Differential interleukin-1 receptor antagonism on pancreatic beta and alpha cells. Studies in rodent and human islets and in normal rats. Diabetologia 36: 759–766
32. Andersen HU, Mandrup-Poulsen T, Egebjerg J, Helqvist S, Nerup J (1989) Genetically determined differences in newborn rat islet sensitivity to interleukin-1 in vitro: no association with the diabetes prone phenotype in the BB-rat. Acta Endocrinol (Copenh) 120: 92–98
33. Spinas GA, Mandrup-Poulsen T, Mølvig J et al. (1986) Low concentrations of interleukin-1 stimulate and high concentrations inhibit insulin release from isolated rat islets of Langerhans. Acta Endocrinol (Copenh) 113: 551–558
34. Palmer JP, Helqvist S, Spinas GA et al. (1989) Interaction of β-cell activity and IL-1 concentration and exposure time in isolated rat islets of Langerhans. Diabetes 38: 1211–1216
35. Helqvist S, Bouchelouche PN, Johannesen J, Nerup J (1990) Interleukin 1β increases the cytosolic free sodium concentration in isolated rat islets of Langerhans. Scand J Immunol 32: 53–58
36. Mandrup-Poulsen T, Spinas GA, Prowse SJ et al. (1987) Islet cytotoxicity of interleukin 1: influence of culture conditions and islet donor characteristics. Diabetes 36: 641–647
37. Sinkowitch L (1985) Intracellular pH modulates the generation of superoxide radicals by human neutrophils. J Clin Invest 76: 1079–1089
38. Helqvist S, Polla BS, Johannesen J, Nerup J (1991) Heat shock protein induction in rat pancreatic islets by recombinant human interleukin 1β. Diabetologia 34: 150–156
39. Asayama K, Kooy NW, Burr IM (1986) Effect of vitamin E deficiency and selenium deficiency on insulin secretory reserve and free radical scavenging systems in islets; decrease of islet manganosuperoxide dismutase. J Clin Lab Med 107: 464–495
40. Sumoski W, Baquerizo H, Rabinovitch A (1989) Oxygen free radical scavengers protect rat islet cells from damage by cytokines. Diabetologia 32: 792–796
41. Southern C, Schulster D, Green IC (1990) Inhibition of insulin secretion by interleukin-1β and tumor necrosis factor-α via an L-arginine-dependent nitric oxide generating mechanism. FEBS lett 276: 42–44
42. Fehsel K, Jalowy A, Qi S, Burkart V, Hartmann B, Kolb H (1993) Islet cell DNA is a target of inflammatory attack by nitric oxide. Diabetes 42: 496–500
43. Marletta MA (1993) Nitric oxide synthase structure and mechanism. J Biol Chem 268: 12231–12234
44. Lyons CR, Orloff GJ, Cunningham JM (1992) Molecular cloning and functional expression of an inducible nitric oxide synthase from a murine macrophage cell line. J Biol Chem 267: 6370–6374
45. Stadler J, Stefanovic-Racic M, Billiar TR et al. (1991) Articular chondrocytes synthesize nitric oxide in response to cytokines and lipopolysaccharide. J Immunol 147: 3915–3920
46. Beasley D, Schwartz JH, Brenner BM (1991) Interleukin 1 induces prolonged L-arginine-dependent cyclic guanosine monophosphate and nitrite production in rat vascular smooth muscle cells. J Clin Invest 87: 602–608
47. Andersen HU, Zumsteg U, Mose Larsen P et al. (1992) 2-D protein map of islets of Langerhans: effects of interleukin-1β and nicotinamide. Diabetologia 35 [Suppl 1]: A212 (Abstract)
48. Karlsen AE, Boel E, Cuartero BG et al. (1993) Cloning of an IL-1 inducible nitric oxide synthase isoform from rat islets also detected in cytokine exposed human islets and RIN cells. Endothelium 1 [Suppl]: S26
49. Gerling IC, Karlsen AE, Champman HD et al. (1994) The inducible nitric oxide synthase gene, Nos2, maps to mouse chromosome 11. Mammalian Genome, 5: 318–320
50. Kolb H, Kolb-Bachofen V (1992) Type 1 (insulin-dependent) diabetes mellitus and nitric oxide. Diabetologia 35: 796–797
51. Mandrup-Poulsen T, Corbett JA, McDaniel ML, Nerup J (1993) What are the types and cellular sources of free radicals in the pathogenesis of type 1 (insulin-dependent) diabetes mellitus? Diabetologia 36: 470–471
52. Kröncke K-D, Funda J, Berschick B, Kolb-Bachofen V (1991) Macrophage cytotoxicity towards isolated rat islet cells: neither lysis nor its protection by nicotinamide are beta-cell specific. Diabetologia 34: 232–238

53. Corbett JA, Wang JL, Sweetland MA, Lancaster Jr. JR, McDaniel ML (1992) IL-1β induces the formation of nitric oxide by β-cells purified from rodent islets of Langerhans: evidence for the β-cell as a source and site of action of nitric oxide. J Clin Invest 90: 2384–2391
54. Kleeman R, Rothe H, Kolb-Bachofen V et al. (1993) Transcription and translation of inducible nitric oxide synthase in the pancreas of prediabetic BB rats. FEBS lett 328: 9–12
55. Andersen HU, Jørgensen KH, Egeberg J, Mandrup-Poulsen T, Nerup J (1993) Nicotinamide reduces interleukin-1-induced nitric oxide production and inhibition of insulin release in rat islets of Langerhans. Acta Endocrinol (Copenh) 128 [Suppl 1] 4
56. Hammonds P, Beggs M, Beresford G, Espinal J, Clarke J, Mertz RJ (1990) Insulin-secreting β-cells possess specific receptors for interleukin-1β. FEBS lett 261: 97–100
57. Brawn K, Fridovich I (1981) DNA strand scission by enzymatically generated oxygen radicals. Arch Biochem Biophys 206: 414–419
58. Corbett JA, Lancaster Jr. JR, Sweetland MA, McDaniel ML (1991) Interleukin-1β-induced formation of EPR-detectable iron-nitrosyl complexes in islets of Langerhans. Role of nitric oxide in interleukin-1β-induced inhibition of insulin secretion. J Biol Chem 266: 21351–21354
59. Welsh N, Eizirik DL, Bendtzen K, Sandler S (1991) Interleukin-1β-induced nitric oxide production in isolated rat pancreatic islets requires gene transcription and may lead to inhibition of the Krebs cycle enzyme aconitase. Endocrinology 129: 3167–3173
60. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA (1990) Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci (USA) 87: 1620–1624
61. Beckman JS, Carson M, Smith CD, Koppenol WH (1993) ALS, SOD and peroxynitrite. Nature 364: 584
62. Ghosh S, Palmer SM, Rodrigues NR et al. (1993) Polygenic control of autoimmune diabetes in nonobese diabetic mice. Nature Genetics 4: 404–409
63. Crisa L, Mardes JP, Rossini AA (1992) Autoimmune diabetes in the BB-rat. Diabetes Metab Rev 8: 9–37