Diabetologia (1997) 40: 352–356

Diabetologia

For debates

The GABA network and the pathogenesis of IDDM

M. Degli Esposti, I. R. Mackay

Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia

Insulin-dependent diabetes mellitus (IDDM) is caused by a lymphocyte-mediated autoimmune destruction of pancreatic beta cells [1, 2]. Another cause of IDDM that is relevant to our present proposal is deficient mitochondrial function of beta cells due to lesions in mitochondrial DNA [3, 4]. Autoimmune IDDM can occur either acutely in childhood, or gradually as latent autoimmune diabetes of adults [5]. Of note is the recent unexplained increase in IDDM incidence, in particular in European countries [6] and also globally [7].

The origins of autoimmunity, reflecting a failure of natural immune tolerance to self constituents of the body (autoantigens), have been long debated, for IDDM in particular [1, 8] and for autoimmune disease in general [9, 10]. Genotype is important, in that an age-dependent high risk is conferred by an appropriate constellation of susceptibility alleles, particularly those derived from the major histocompatibility complex (MHC) [1, 8, 11], specified as *idd*[1] [12]. Risk is also conferred by various non-MHC background genes [13]. As judged from IDDM-specific serum autoantibodies [14], the major autoantigens of the beta cell include glutamic acid decarboxylase (GAD) [15, 16], insulin [17], and a molecule called islet cell antigen 512 [18, 19] or IA-2 [20], which is probably a protein tyrosine phosphatase corresponding to the 37/40 kDa autoantigen [21, 22]. Overall, anti-GAD is the most frequent autoimmune reactivity in IDDM [15, 23], and, together with anti-ICA512, accounts for most of the islet cell antibodies (ICA) demonstrable by immunofluorescence [19]. Anti-GAD is also claimed to be the earliest antibody generated, at least in non-obese diabetic (NOD) mice [24].

Various hypotheses have been proposed regarding the provocation of the autoimmune process leading to IDDM in the high-risk genotype. One favoured idea is based on molecular mimicry [1, 23, 25, 26] whereby the critical event is infection with a virus or micro-organism that displays to the immune system a protein resembling a constituent of the body sufficiently closely so that the response is cross-reactive with an autologous molecule (self). The coxsackie B4 virus has been particularly incriminated in IDDM, according to seasonality of the disease onset; sequence similarity between GAD and virus proteins [1, 23, 25]; identification of viral sequences in serum of children at onset of disease [27]; and a high frequency of serological evidence of enterovirus exposure both in utero and in childhood [28]. On the other hand, the concept of molecular mimicry is not sustained by the failure of monoclonal antibodies to recognize sequences of GAD [29], nor does it explain why autoimmune responses to GAD specifically occur in beta cells. Thus, even if enterovirus infections were relevant to the pathogenesis of IDDM, mechanisms other than molecular mimicry could be involved.

We suggest that the pathogenesis of IDDM depends on an immunological response to a critical natural autoantigen that is up-regulated and abnormally presented to the immune system by metabolic alterations or islet cell damage. This idea has been considered previously [30], but has not been developed as a credible alternative to molecular mimicry. Here we describe biochemical pathways for the metabolic induction and up-regulation of the autoantigen GAD, and how this might sustain the specific autoimmune

Corresponding author: Dr. M. Degli Esposti, Department of Biochemistry and Molecular Biology, Monash University, Clayton, 3168 Victoria, Australia

Abbreviations: IDDM, Insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; Oxphos, mitochondrial oxidative phosphorylation; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; ICA, islet cell antibodies; TCA, tricarboxylic acid.

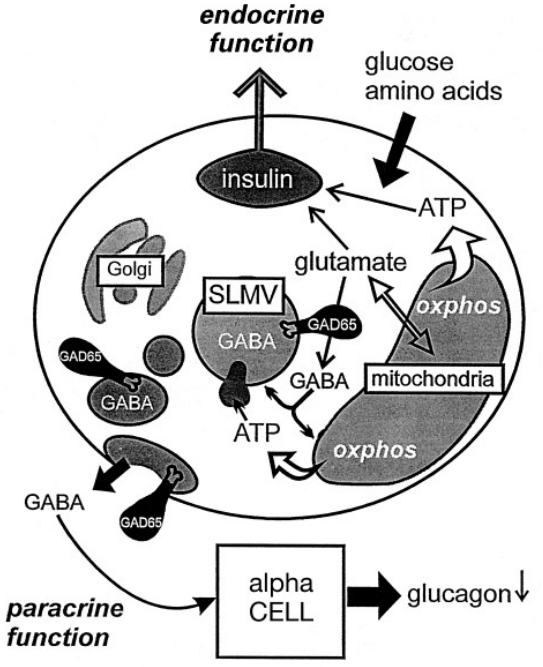

Fig. 1. Mitochondrial oxidative phosphorylation, the GABA network and up-regulation of GAD in beta cells. The beta-cell subserves insulin release, and secretion of GABA via the synaptic-like-microvesicles (SLMV), to modulate the secretion of glucagon by alpha cells. These activities depend on GABA and ATP, requiring full functional activity of mitochondrial oxidative phosphorylation (oxphos). Oxphos provides ATP for insulin release and for transporting GABA into the SLMV by a proton-ATPase. GAD65, the predominant and antigenic form of GAD, is bound to these vesicles and thereby transferred to the plasma membrane by exocytosis with release of GABA. Metabolic perturbation of the GABA network, e. g. by impairment of oxphos, leads to up-regulation of GAD and its increased expression at the cell surface via vesicle traffic and exocytosis

destruction of beta cells and the eventual development of IDDM. These pathways depend critically on mitochondrial oxidative phosphorylation (oxphos), and include key metabolic interactions of γ-aminobutyric acid (GABA) that we designate the *GABA network* of the beta cell.

Critical effects of oxidative phosphorylation deficiency

Islet cells have many similarities to neurons [30, 31], one being their high energy dependence on mitochondrial oxphos [31]. In particular, beta cells require mitochondrial ATP to sustain the production and release of insulin [31–34]. Indeed, the increase of intracellular ATP which is stimulated by a high blood glucose level is the signal for insulin release via a block of potassium channels in the plasma membrane of the beta cells [32–34]. The critical role of oxphos in providing this ATP signalling is clearly illustrated by the high susceptibility of beta cells to energy deficits consequent to mitochondrial gene mutations [3, 4], now recognized as the disease entity 'mitochondrial diabetes' [4]. We consider that various toxins, febrile states or even sugar overload can induce a deficiency in mitochondrial oxphos similar to that resulting from inherited mutations, albeit only transiently. This environmentally induced oxphos deficiency will then perturb the GABA network of beta cells.

Perturbation of the GABA network

The GABA network of the beta cell describes crucial biochemical pathways, central to which is the neurotransmitter GABA, itself the product of the catalytic activity of GAD. As mentioned, beta cells resemble neurons in many respects [30, 31], including their strict dependence on oxphos and also the high content GABA of which they secrete via synaptic-like microvesicles [35, 36]. The increased ATP levels produced by accelerated oxphos in response to high blood glucose levels not only promote insulin release [32–34], but also facilitate the accumulation of GABA into synaptic-like microvesicles and its subsequent release within the islets [35, 36]. This release of GABA fulfills the crucial paracrine function of fine regulation of the secretion of other islet cell hormones, especially glucagon [37] (Fig. 1). Since all of the ATP in beta cells is produced by mitochondrial oxphos [31], the endocrine function of insulin secretion, and the paracrine function of GABA secretion, are both strictly dependent on full functional activity of mitochondrial oxphos.

In addition, the GABA network and oxphos are intimately connected in the beta cells by a mitochondrial enzyme, GABA transaminase [38], which directs the catabolism of GABA to produce succinate. Succinate then enters the tricarboxylic acid (TCA) cycle and is directly oxidized by the mitochondrial respiratory chain [38, 39]. The mitochondrial catabolism of GABA, known as the GABA shunt [38], can generate succinate while removing α-ketoglutarate, thus effectively bypassing the dehydrogenases of the TCA cycle, in particular isocitrate dehydrogenase, which are inhibited by high levels of ATP. This bypass could be critical for sustaining the preferential activation of mitochondrial oxphos during a glucose stimulus for insulin release [31–34, 38], and would need to be strictly regulated within the beta cells to maintain an optimal equilibrium between GABA catabolism and GABA release for paracrine signalling (Fig. 1).

Given that the activity of GAD is the rate-limiting step of the GABA shunt [39], GAD can be seen as the key enzyme of the GABA network, to maintain insulin secretion in response to blood glucose and the paracrine function of GABA within the islets (Fig. 1). Consequently, beta cells must compensate

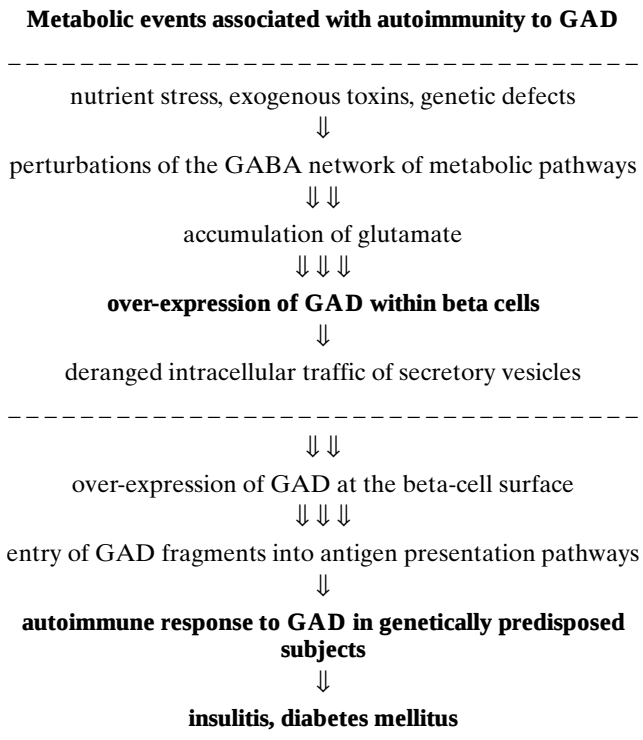

Fig. 2. Proposed sequence of events leading to up-regulation of GAD and the initiation of autoimmunity in beta cells. The upper section itemizes the metabolic steps relevant to IDDM that occur specifically within beta cells, as shown in Figure 1, and the lower section itemizes the immunological steps that lead to insulitis and IDDM

for any perturbation in the GABA network by controlling the activity of GAD. Finally, the resemblance between beta cells and neurons prompts reference to studies on oxidative damage to the brain, e.g. hypoxia [40], wherein the expression and activity of GAD increase in response to the ensuing excess of glutamate that results from impaired mitochondrial respiration. Glutamate inevitably accumulates in cells with a defective respiratory chain because NADH produced in mitochondria cannot be re-oxidized and, consequently, the equilibrium of glutamate dehydrogenase is shifted towards the production of glutamate. Accordingly, in beta cells as in the brain, febrile, hypoxic or environmental toxic stresses would be prejudicial to mitochondrial bioenergetic functions, and to GABA metabolism. The result is over-production of GAD within the cell.

Perturbation of the GABA network in the pathogenesis of IDDM

The concept central to our proposition is that beta cells respond to an excess glutamate accumulation, of toxic, metabolic or other origin, with an over-expression of GAD. This up-regulation would also coordinately maintain the high levels of GABA required for both its energetic [38, 39] and paracrine functions. In turn, over-expression of GAD will influence the cellular traffic of synaptic-like microvesicles, and so result in an increased extracellular presentation of GAD65, the prevalent isoform of GAD in human beta cells which is associated with synaptic-like microvesicles [15, 23, 35] (Fig. 1).

This enhanced intracellular expression of GAD, and its presumed presentation at the beta-cell surface, will perturb tolerance to it in individuals with particular genotypes, and thus initiate the autoimmune cascade leading to IDDM. Supporting this, studies on animal models of IDDM suggest that a link exists between the metabolic state of beta cells and their expression of cell surface antigens [1, 41]. Indeed, in NOD mice anti-GAD is the first of several autoantigenic responses [24], and there is also an increased islet cell expression of GAD and GABA [42]. Thus, in mice as in humans, the metabolic link could be provided by the GABA network and its regulation to preserve paracrine function in the islets.

The steps from over-expression of the GAD autoantigen to the immunological initiation of destructive insulitis remain hypothetical. It should be considered how GAD, or other islet cell autoantigens, enter the requisite pathways for presentation by MHC molecules to T lymphocytes. Presumably, the high-risk genotype will allow for the presence in peripheral tissues of T lymphocytes with a capability for reactivity with appropriately expressed epitopes of GAD. Intracellular over-expression of GAD could, in the predisposing genotype, result in efficient presentation of GAD fragments at the cell surface through the class I pathway and engagement of CD8 cytotoxic T lymphocytes, as shown in the NOD mouse [43]. Alternatively, promiscuous extracellular presentation of GAD, or incidental accompanying damage to beta cells with release of intracellular GAD, could allow for the uptake of GAD by the macrophage-dendritic types of antigen-presenting cells normally present within the islets [44], and their presentation of autoantigenic moieties to CD4 T lymphocytes. We note that prominence of macrophage-type cells in islets is one of the earliest features of the insulitis lesion of IDDM [44, 45]. Once CD4 or CD8 T cells with specificity for GAD are recruited to the islets, the stage is set for an influx of other immune inflammatory cell types and activation of downstream events including release of cytokines characteristic of inflammatory autoimmune responses [1, 46].

The steps whereby beta cells become specific targets for GAD autoimmunity are shown in Figure 2. One or more defects in the energy and glutamate metabolism in beta cells would induce an over-expression of GAD. This would also increase the breakdown of GAD and the recycling of its degradation fragments, thus overloading the MHC system and creating the conditions for T-cell recognition and

autoimmune insulitis targeted at beta cells that culminates in IDDM. Thus, a perturbation of the GABA network results in an enhanced presentation of potentially immunogenic GAD at the surface of beta cells.

Wider implications of metabolic induction of IDDM

The recent discovery that the rodenticide Vacor, a potent environmental inducer of IDDM in humans, specifically inhibits mitochondrial respiration [47] provides further evidence in support of the concept that mitochondrial malfunction can be relevant to the pathogenesis of diabetes.

Our proposition of a metabolic induction of IDDM by toxic exposure or nutrient stress would explain the 'random' component in disease susceptibility [1], as well as having wider implications for the induction of autoimmunity in general, together with important practical connotations. In particular, the increased incidence of IDDM in recent decades with its apparent correlation with changing dietary habits, including excessive loads of sugars and amino acids [1], readily fits in with our proposal. Additionally, the cellular level of critical autoantigens has been suggested to influence the expression of autoimmune diseases including IDDM [1], since when such an expression is alleviated there is a well-recognized beneficial effect, initially called "beta-cell rest" [48], which has been recently validated experimentally [41, 49]. In the context of our ideas, this effect could be rationalized as a result of exogenously induced relief of metabolic stress on beta cells under the 'pressure' of high blood glucose in individuals with an imbalanced GABA network. This concept has also been applied to the treatment of autoimmune hyperthyroidism by thyroxine to reduce thyroid antigen release [50].

Finally, dietary deficiency of crucial amino acids is believed to contribute to the pathogenesis of non-insulin-dependent diabetes mellitus (NIDDM) [51], and the present proposal could provide a biochemical rationale to link the metabolism of the central amino acid glutamate to the unique cellular activity of pancreatic beta cells.

Conclusions

The GABA network concept could represent a unifying scheme for key metabolic defects relevant to IDDM and also to some types of NIDDM including mitochondrial diabetes [4]. In fact, the essential difference between IDDM and such types of NIDDM could simply be the superimposition of the autoimmune response, itself linked to multiple genetic elements, which accelerates the destruction of the beta cells. The state sufficient for IDDM pathogenesis is a susceptible genotype and an overproduction of an intrinsic autoantigen, GAD, as a consequence of abnormalities in crucial metabolic pathways that are specific to the beta cells of pancreatic islets, the GABA network. This conclusion may encourage further debate.

Acknowledgements. Professors A. W. Linnane and P. Z. Zimmet, and Drs. M. J. Rowley and M. A. Myers contributed helpful discussion and critique. We thank B. and R. Grollo for financial support.

References

1. Atkinson MA, Maclaren NK (1994) The pathogenesis of insulin-dependent diabetes mellitus. New Engl J Med 331: 1428–1436
2. Bach JF (1995) Organ-specific autoimmunity. Immunol Today 16: 353–355
3. Gerbitz KD (1992) Does the mitochondrial DNA play a role in the pathogenesis of diabetes? Diabetologia 35: 1181–1186
4. Gerbitz KD, Van den Ouweland JMW, Maassen JA, Jaksh M (1995) Mitochondrial diabetes mellitus: a review. Biochim Biophys Acta 1271: 253–260
5. Tuomi T, Groop LC, Zimmet PZ et al. (1993) Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. Diabetes 42: 359–362
6. Drykoningen CEM, Mulder ALM, Vaandrager GJ, LaPorte RE, Bruining GJ (1992) The incidence of male childhood type 1 (insulin-dependent) diabetes mellitus is rising rapidly in the Netherlands. Diabetologia 35: 139–142
7. Diabetes Epidemiology Research International Mortality Group (1991) Major cross-country differences in risk of dying for people with IDDM. Diabetes Care 14: 49–54
8. Hagopian W, Lernmark A (1992) Autoimmune diabetes mellitus. In: Rose NR, Mackay IR, (eds) The autoimmune disease II. Academic Press, San Diego, pp 235–278
9. Lanzavecchia A (1995) How can cryptic epitopes trigger autoimmunity? J Exp Med 181: 1945–1948
10. Behar S, Porcelli SA (1995) Mechanisms of autoimmune disease induction. Arthritis Rheumatism 38: 458–476
11. Caillat-Zucman S, Garchon H-J, Timsit J et al. (1992) Age-dependent HLA genetic heterogeneity of type 1 insulin-dependent diabetes mellitus. J Clin Invest 90: 2242–2250
12. Baxter AG, Cooke A (1995) The genetics of the NOD mouse. Diab Metab Rev 11: 315–335
13. Davies JK, Kawaguchi Y, Bennett ST et al. (1994) A genome-wide search for human type 1 diabetes susceptibility genes. Nature 371: 130–136
14. Hagopian WA, Sanjeevi CB, Kockum I et al. (1995) Glutamate decarboxylase-, insulin-, and islet cell-antibodies and HLA typing to detect diabetes in general population-bases of Swedish children. J Clin Invest 95: 1505–1511
15. Baekkeskov S, Aanstoot H-J, Christgau S et al. (1990) Identification of the 64k autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 347: 151–156
16. Myers MA, Mackay IR, Zimmet PZ, Rowley MJ (1996) Autoantibodies to glutamate decarboxylase in insulin-dependent diabetes mellitus. Diabetes Annual 10: 15–36
17. Palmer JP (1993) Predicting IDDM. Diabetes Rev 1: 104–115

18. Rabin DU, Pleasic SM, Shapiro JA et al. (1994) Islet cell antigen 512 is a diabetes-specific islet autoantigen related to protein tyrosine phosphatases. J Immunol 152: 3183–3188
19. Myers MA, Rabin DU, Rowley MJ (1995) Pancreatic islet cell cytoplasmic antibody in diabetes is represented by antibodies to islet cell antigen 512 and glutamic acid decarboxylase. Diabetes 44: 1290–1295
20. Lan MS, Lu J, Goto Y, Notkins AL (1994) Molecular cloning and identification of a receptor-type protein tyrosine phosphatase, IA-2, from human insulinoma DNA. Cell Biol 13: 505–514
21. Bonifacio E, Lampasona V, Genovese S, Ferrari M, Bosi E (1995) Identification of protein tyrosine phosphatase-like IA2 (islet cell antigen 512) as the insulin-dependent diabetes-related 37/40K autoantigen and a target of islet-cell antibodies. J Immunol 155: 5419–5426
22. Passini N, Larigan JD, Genovese S, Appella E, Sinigaglia F, Rogge L (1995) The 37/40-kilodalton autoantigen in insulin-dependent diabetes mellitus is the putative tyrosine phosphatase IA-2. Proc Natl Acad Sci USA 92: 9412–9416
23. Solimena M, De Camilli P (1995) Coxsackieviruses and diabetes. Nature Medicine 1: 25–26
24. Tisch R, Yang X-D, Singer SM et al. (1993) Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. Nature 366: 72–75
25. Kaufman DL, Erlander MG, Clare-Salzler M, Atkinson MA, Maclaren NK, Tobin AJ (1992) Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent diabetes mellitus. J Clin Invest 89: 283–292
26. Baum H, Butler P, Davies H, Sternberg M, Burroughs AK (1993) Autoimmune disease and molecular mimicry: an hypothesis. Trends Biochem Sci 18: 140–144
27. Clements GB, Galbraith DN, Taylor KW (1995) Coxsackie B virus and onset of childhood diabetes. Lancet 346: 221–223
28. Hyoty H, Hiltunen M, Knip M et al. (1995) A prospective study of the role of coxsackie B and other enterovirus infections in the pathogenesis of IDDM. Diabetes 44: 652–657
29. Richter W, Mertens T, Schoel B et al. (1994) Sequence homology of the diabetes-associated autoantigen glutamate decarboxylase with coxsackie B2–2C protein and heat shock protein 60 mediates no molecular mimicry of autoantibodies. J Exp Med 180: 721–726
30. Atkinson MA, Maclaren NK (1993) Islet cell autoantigens in insulin-dependent diabetes. J Clin Invest 92: 1608–1616
31. Erecinska M, Bryla J, Michalik M, Meglasson MD, Nelson D (1992) Energy metabolism in islets of Langerhans. Biochim Biophys Acta 1101: 273–295
32. Malaisse WJ (1992) Glucose-sensing by pancreatic B-cell: the mitochondrial part. Int J Biochem 24: 693–701
33. Duchen MR, Smith PA, Ashcroft FM (1993) Substrate-dependent changes in mitochondrial function, intracellular free calcium and membrane channels in pancreatic β cells. Biochem J 294: 35–42
34. Sener A, Malaisse WJ (1987) Stimulation by D-glucose of mitochondrial oxidative events in islet cells. Biochem J 246: 89–95
35. Reetz A, Solimena M, Matteoli M, Folli F, Takei K, De Camilli P (1991) GABA and pancreatic β cells: colocalization of glutamic acid decarboxylase (GAD) and GABA with synaptic-like microvesicles suggests their role in GABA storage and secretion. EMBO J 10: 1275–1284
36. Thomas-Reetz A, Hell JW, Duenzymesring MJ, Walch-Solimena C, Jahn R, De Camilli P (1993) γ-aminobutyric acid transporter driven by a proton pump is present in synaptic-like microvesicles of pancreatic β cells. Proc Natl Acad Sci USA 90: 5317–5321
37. Rorsman P, Berggren P, Bokvist K et al. (1989) Glucose-inhibition of glucagon secretion involves activation of $GABA_A$-receptor chloride channels. Nature 341: 233–236
38. Sorenson RB, Garry DG, Brelje CT (1991) Structural and functional considerations of GABA in islets of Langerhans. Diabetes 40: 1365–1374
39. Michalik M, Nelson J, Erecinska M (1993) GABA production in rat islets of Langerhans. Diabetes 42: 1506–1513
40. Romijn HJ, Janszen AWJW, Van den Bogert C (1994) Permanent increase of immunocytochemical reactivity for γ-aminobutyric acid (GABA), glutamic acid decarboxylase, mitochondrial enzymes, and glial fibrillary acidic protein in rat cerebral cortex damaged by early postnatal hypoxia-ischemia. Acta Neuropathol 87: 612–627
41. Aaen K, Rygaard J, Josefsen K et al. (1990) Dependence of antigen expression on functional state of β-cells. Diabetes 39: 697–701
42. Martignat L, Elmansour A, Audrain M, Julien JF, Charbonnel B, Sai P (1995) Pancreatic expression of antigens for islet cell antibodies in non-obese diabetic mice. J Autoimmun 8: 465–482
43. Nagata M, Santamaria P, Kawamura T, Utsugi T, Yoon J-W (1994) Evidence for the role of $CD8^+$ cytotoxic T cells in the destruction of pancreatic β-cells in nonobese diabetic mice. J Immunol 152: 2042–2050
44. Jansen A, Homo-Delarche F, Hooijkaas H et al. (1994) Immunohistochemical characterization of monocytes-macrophages and dendrite cells involved in the initiation of insulitis and β-cell destruction in NOD mice. Diabetes 43: 667–675
45. Lee KU, Amano K, Yoon J-W (1988) Evidence for initial involvement of macrophage in development of insulitis in NOD mice. Diabetes 37: 989–991
46. Shehadeh NN, LaRosa F, Lafferty KJ (1993) Altered cytokine activity in adjuvant inhibition of autoimmune diabetes. J Autoimmun 6: 291–300
47. Degli Esposti M, Ngo A, Myers MA (1996) Inhibition of mitochondrial complex I may account for IDDM induced by intoxication with the rodenticide Vacor. Diabetes 45: 1531–1534
48. Ludvigsson J, Heding LG, Larsson Y, Leander E (1977) C-peptide in juvenile diabetics beyond the post-initial remission period. Relation to clinical manifestation at onset of diabetes, remission and diabetic control. Acta Pediatr Scand 66: 177–184
49. Bjork E, Kampe O, Andersson A, Karlsson FA (1992) Expression of the 64 kDa/glutamic acid decarboxylase rat islet cell autoantigen is influenced by the rate of insulin secretion. Diabetologia 32: 490–493
50. Hashizume K, Ichikawa K, Sakurai A et al. (1991) Administration of thyroxine in treated Graves' disease. New Engl J Med 314: 947–953
51. Hales CN, Barker DJP (1992) Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 35: 595–601