Neuroendocrine Immuno-ontogeny of the Pathogenesis of Autoimmune Diabetes in the Nonobese Diabetic (NOD) Mouse

Françoise Homo-Delarche

Abstract

Type 1 diabetes (T1D) is a T cell-mediated autoimmune disease in which insulin-producing beta cells of the pancreatic islets of Langerhans are destroyed. The nonobese diabetic (NOD) mouse is one of the rare spontaneous models that enable the study of prediabetic pancreatic events. The etiology of the autoimmune attack in human and animal T1D is still unknown, but genetic and environmental factors are involved in both cases. Although several autoantigens have been identified, and defective immune-system regulation is implicated, this information does not satisfactorily explain the generally accepted beta-cell specificity of the disease or how so many and diverse environmental factors intervene in its pathogenesis. Based on data obtained from evaluating glucose homeostasis in a variety of situations, particularly stress and cytokine administration, in young prediabetic NOD mice, the author hypothesizes that the islet of Langerhans is a major actor, and its altered regulation through environmentally induced insulin resistance might reveal latent T1D. It is also postulated that T1D pathogenesis might be linked to abnormal pancreas development, probably due to disturbances of glutamic acid decarboxylase (GAD)\(^1\) innervation phagocytosis by defective macrophages during the early postnatal period. Also discussed is the role of defective presentation of pancreatic hormones and GAD in the thymus, and its potential repercussion on T-cell tolerance. Observations have demonstrated that the diabetogenic process in the NOD mouse is extremely complex, involving neuroendocrine immune interaction from fetal life onward.

Key Words: androgens; beta-cell hyperactivity; cytokines; glucocorticoids; inflammation; insulin resistance; nonobese diabetic mouse; type 1 diabetes

Introduction

Type 1 diabetes mellitus (T1D\(^1\)), observed predominantly in children and young adults, is a multigenic disease strongly dependent on numerous environmental factors, as schematized in Figure 1 (Atkinson and Eisenbarth 2001; Dahlquist et al. 1999; Homo-Delarche 1997; Todd and Wicker 2001). T1D results from the autoimmune destruction of insulin-producing beta cells in pancreatic islets of Langerhans. Despite extensive genetic and immunological investigations, mainly conducted in spontaneous animal models (particularly the nonobese diabetic [NOD\(^1\)] mouse but also the bio-breeding [BB\(^1\)] rat), the etiology of the disease remains unknown (Atkinson and Eisenbarth 1999; Mathis et al. 2001; Notkins and Lernmark 2001). As in humans, autoimmune diabetes is a polygenic disorder in which more than 15 susceptibility loci have been described (Todd and Wicker 2001). Because genetic and immunological data have been abundantly reviewed (Atkinson and Eisenbarth 2001; Atkinson and Leiter 1999; Bach 1994; Beyan et al. 2003; Moriyama and Eisenbarth 2002; Todd 1995; Todd and Wicker 2001), the focus in this article is on the less known neuroendocrine aspects of diabetes development in the prediabetic NOD mouse.

In NOD mice, the first immune cells, macrophages (M\(\Phi_s\)) and dendritic cells (DCs\(^1\)), also known as antigen-presenting cells (APCs\(^1\)), accumulate around NOD pancreatic islets and ducts at weaning (3 wk of age) (Figure 2) (Jansen et al. 1994; Jansen et al. 1996; Rosmalen et al. 2002a). Subsequently, T cells migrate to the pancreas and home (or accumulate) around ducts (periductulitis) and islets (peri-insulitis), followed by M\(\Phi\), DC, and T-cell infiltration into the islets (insulitis). Destructive insulitis coincides with scavenger M\(\Phi\) influx and finally leads to beta-cell destruction and overt symptoms of T1D (Jansen et al. 1994; Rosmalen et al. 2002a). Generally, NOD females, whose islets are precociously infiltrated, become diabetic earlier (as of 3 mo of age) and at greater frequency than males. In the Necker Hospital colony (Paris, France), 80% and 40% of females and males, respectively, generally become diabetic at 6 mo of age.

Because of the existence of NOD sexual dimorphism and the impact of some environmental factors (e.g., stress, infections, and diet) on the clinical onset of T1D, colleagues

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\(^1\)Abbreviations used in this article: APC, antigen-presenting cell; BB, bio-breeding; CBG, corticosteroid-binding globulin; DC, dendritic cell; EFAD, essential fatty acid deficient; GABA, \(\gamma\)-aminobutyric acid; GAD, glutamic acid decarboxylase; GFAP, glial fibrillary acidic protein; HPA, hypothalamus-pituitary-adrenal; IAA, insulin autoantibody; IFN\(\gamma\), interferon-\(\gamma\); IGF-1, insulin growth factor-1; IL, interleukin; LADA, latent autoimmune diabetes of adults; MHC, major histocompatibility complex; M\(\Phi\), macrophage; NOD, nonobese diabetic; NON, nonobese nondiabetic; NOR, nonobese resistant; Sc, Schwann cell; T1D, type 1 diabetes; T2D, type 2 diabetes; TNF, tumor necrosis factor; TZD, thiazolidinedione; VNTR, variable number of tandem repeats.
and I examined more closely the impact of some of these factors on NOD mice. The data obtained led us to study glucose homeostasis in young prediabetic NOD mice, and we advanced the hypothesis that the islet of Langerhans and its innervation are major actors in T1D progression (Homo-Delarche 1997; Homo-Delarche and Boitard 1996; Homo-Delarche 1997). Moreover, in the context of the possible role of defective presentation of pancreatic hormones in the thymus with one or more potential repercussions on T-cell tolerance, we also evaluated the thymic expression of pancreatic hormones. Our own data presented in this article are interwoven with those of other investigators obtained in similar or close domains to yield a coherent working hypothesis of T1D pathogenesis. Furthermore, as often as possible, we compare the neuroendocrine events that take place during the progression of the NOD disease with human T1D.

Effects of Hormones and Environment on NOD Insulitis and Diabetes Incidence

Hormones and environmental factors modulate immune and autoimmune responses (Homo-Delarche and Dardenne 1993; Homo-Delarche and Durant 1994). Sex steroids are part of the mechanism underlying the well-recognized immune sexual dimorphism, which is particularly evident in autoimmune diseases (Ansar Ahmed et al. 1985; Talal and Ansar Ahmed 1987). Moreover, clinical and experimental observations link stressful life events and the onset of these diseases (Homo-Delarche et al. 1991). As part of the endocrine response to stress, glucocorticoids exert well-known anti-inflammatory and immunosuppressive actions but also act as counterregulatory hormones inducing hyperglycemia (Homo-Delarche et al. 1991; Porte and Woods 1983). Therefore, in T1D, glucocorticoids might have both potentially beneficial and deleterious effects. Moreover, NOD diabetes can be modulated not only by multiple immunotherapeutic agents (Bach 2002b), but also by various other factors, including melatonin, ambient temperature, variations of protein and carbohydrate intake, insulin and drugs modulating its secretion or sensitivity, insulin growth factor-1 (IGF-1), and leptin.

Sex Steroids, Pregnancy, Prolactin, and Vitamin D

As mentioned above, NOD mice show sexual dimorphism, with diabetes appearing earlier and more often in females than males in most colonies. Orchidectomy soon after weaning significantly increases the prevalence of the disease,


Figure 2 Sequential appearance of antigen-presenting cells (APCs), both dendritic cells and macrophages, and lymphocytes in nonobese diabetic (NOD) type 1 diabetes mouse pancreata. Clinical onset of diabetes appears when more than 80% of the islets evidence insulitis.

2Allusions in this article to the author’s work with others relate to research with colleagues in their laboratory at CNRS UMR 8603, Hospital Necker/Université Paris 5, Paris, France.
whereas ovariectomy reduces it in young adults (Fitzpatrick et al. 1991; Hawkins et al. 1993; Makino et al. 1981; Verheul et al. 1995). Generally in those studies, the degree of insulitis coincided with progression to diabetes. Androgen (dihydrotestosterone) administration, at pharmacological but also near physiological doses, prevents the disease in females, whereas a physiological or near physiological, nontoxic dose of estrogens does not affect disease symptoms, raising the possibility that the NOD sexual dimorphism is linked to the protective immunosuppressive effects of androgens (Fox 1992; Hawkins et al. 1993; Verheul et al. 1995). In terms of circulating sex steroids, when compared with NOD females, NOD males appear, as expected, to differ only in androgen levels (Durant et al. 1998). Intriguingly, although the incidence of diabetes is lower in NOD females treated with androgens from birth onward, ovariectomy + testosterone increases it to 100%, suggesting that the neonatal hormonal environment strongly influences diabetes incidence in NOD mice (Hawkins et al. 1993). To what extent this effect on diabetes incidence results from the alteration of the hypothalamus-pituitary-adrenal (HPA) axis due to ovariectomy (and also sham surgery) performed during the early postnatal period, as demonstrated in rats, is unknown (Vallette et al. 1982). In this setting, glucocorticoid effects all along the HPA axis might be down-regulated, thereby modifying the postnatal increase and functioning of their receptors (Sakly and Koch 1981). Of note, at variance with what is observed in NOD mice, no sexual dimorphism exists in the BB rat (Rossini et al. 1995). In human T1D, a slight male preponderance can be observed, at least in some countries (Homo-Delarche 1997).

The percentages of thymus and spleen cell subpopulations, mitogen reactivity, lymphokine production, and in vivo response to a thymus-dependent antigen (e.g., sheep red blood cells) are dependent on or independent of the sex-steroid environment in adult prediabetic NOD mice. For example, although castration in both sexes does not affect splenic T-cell populations, splenic B cells and Mφs are significantly increased (Fitzpatrick et al. 1991). Moreover, the sexual dimorphism in NOD diabetes incidence (females being more diabetes prone than males) is reflected by a difference in thymocyte apoptosis (Casteels et al. 1998). Apoptosis is decreased 24 hr after incubation with dexamethasone in both sexes of NOD compared with C57BL/6 mice, but was lower in NOD females than in NOD males. Moreover, orchidectomy (which increases this incidence) diminishes thymocyte apoptosis, and androgen treatment of NOD females (which lowers diabetes incidence) restores thymocyte apoptosis to male levels. The active form of vitamin D₃, 1,25-dihydroxyvitamin D₃, which prevents diabetes in NOD mice, was shown to restore apoptosis levels to those of C57BL/6 controls (Casteels et al. 1998; Gregori et al. 2002; Zella and DeLuca 2003).

At the pancreas level, sexual dimorphism in cytokine production is also found during the early stages of insulitis at 4 wk of age (Bao et al. 2002). The level of the TH1 inflammatory cytokine, interferon-γ (IFNγ)¹, is significantly higher in females, in contrast to the TH2 anti-inflammatory cytokine, interleukin (IL)⁴⁻⁴, which is higher in males. Moreover, estrogens enhance the production of IL-12, an inflammatory cytokine, by CD⁴⁺ T cells, whereas androgens lower IFNγ-induced IL-12 production.

It is pertinent that mega-islet formation, which takes place from 6 to 10 wk of age in NOD mice, also shows sexual dimorphism (Rosmalen et al. 2001; Rosmalen et al. 2002a). NOD females spontaneously develop more mega-islets than NOD males, in which orchidectomy further increases their number. More mega-islets in castrated NOD males are associated with more infiltrating immune cells. These mega-islets are always only weakly insulin positive compared with smaller islets, suggesting their lower insulin content, as if they were actively secreting insulin or, by contrast, completely exhausted. It should be noted that castrated lymphocyte-deficient NODscid mice also have enhanced mega-islet formation and DC infiltration, indicating that lymphocytes are not required for these castration-induced effects. The latter data suggest that the sex difference in mega-islet numbers in NOD mice reflects, in part, an organ-specific androgen effect that is independent of lymphocytes.

Pregnancy is associated with depressed inflammatory and immune responses, and increased growth and function of islets of Langerhans (Aerts and Assche 1975; Homo-Delarche and Durant 1994). NOD mice treated for 13 wk with a cocktail of pregnancy hormones, including a glucocorticoid (dexamethasone), progesterone, estradiol, growth hormone, and prolactin, are protected from diabetes and show less insulitis compared with the control group (saline) (Atwater et al. 2002). Intriguingly, prolactin alone appears to have only a partial protective effect on diabetes progression, despite this hormone being known for its immunostimulatory and deleterious properties in various autoimmune diseases, and its mild counterregulatory effect on insulin, particularly during normal pregnancy (Atwater et al. 2002; Durant et al. 1995; Homo-Delarche and Durant 1994). In NOD mice, pituitary implants on day 35 of age enhance prolactin levels and increase the incidence of diabetes in intact males and ovariectomized females. Similarly, metoclopramide, which counteracts the effect of bromocriptine, known to inhibit prolactin secretion, aggravates diabetes. Inversely, bromocriptine protects NOD females when administered daily subcutaneously, but not intraperitoneally (Durant et al. 1995; Hawkins et al. 1994). This difference results from the hyperglycemic effect of intraperitoneal administration of bromocriptine. Finally, prolactin and IL-2 receptors in splenic and pancreatic immunocompetent cell populations appear to be similarly expressed in NOD females and males (Hawkins et al. 1996).

**Behavior, Stress, Glucocorticoids, and Melatonin**

Based on behavioral testing of the numbers of squares crossed and rearings in an open field, Amrani and col-
leagues (1994) concluded that 2-mo-old prediabetic NOD mice of both sexes are more active than their control C57BL/6 counterparts, which were described as a very active mouse strain. It is generally held that the most active mice in the open field are the least emotional. Moreover, the less emotional females become diabetic earlier and the more diabetes-resistant NOD males are more emotional than NOD females (Ader et al. 1991). NOD mice also exhibit increased sensitivity to the behavioral effects (social exploration, locomotor activity, and rearings) of IL-1 injections compared with outbred ICR mice from which the inbred NOD strain originated (Bluthe et al. 1999). These data suggest that emotionality via neuroendocrine changes, possibly linked to the degree of physical activity, may be a mediating factor that modulates diabetes expression (Ader et al. 1991).

NOD mice have been subjected to various stressors prenatally, neonatally, or during early adulthood. Prenatal stress induced by immobilization of the mothers during the last week of gestation accelerates diabetes onset and increases its prevalence in NOD females, whereas it has no effect in NOD males (Saravia-Fernandez et al. 1996a). Neonatal separation of NOD progeny from their mothers has been reported to increase the risk of developing diabetes in both sexes significantly, but to have no effect on insulitis (Dahlquist and Kallen 1997). It has been suggested that the heightened risk could be associated with the induction of metabolic alterations leading to increased peripheral insulin requirements, perhaps in relation to insulin resistance. Short acute stress, induced by a triad of stressors (overcrowding + repeated restraint + anesthesia) applied during the week immediately after weaning reportedly does not affect diabetes expression (Durant et al. 1993). However, when applied later, from the 6th to the 8th week of age, the same combination of stressors has protected NOD mice, particularly males, against diabetes, (Saravia-Fernandez et al. 1996a). Long-term chronic stress, obtained by restraint once a week or overcrowding, significantly protects NOD females (Durant et al. 1993). However, the stress effect on insulitis does not parallel the effects on diabetes incidence for all of the groups described above. It should also be noted that long-term injection of vehicle (0.9% saline) alone is able to delay the appearance and/or decrease the incidence of diabetes in both sexes of NOD mice (Saravia-Fernandez et al. 1996a). A stressful condition such as immobilization increases circulating corticosterone and progesterone concentrations (in both sexes of NOD mice) concomitantly with testosterone decrease in males (Durant et al. 1998). Moreover, rodents, particularly NOD mice, exhibit marked sexual dimorphism in stress-induced corticosterone (Amrani et al. 1996; Durant et al. 1998; Homo-Delarche et al. 1991).

Although NOD females that have been subjected to repeated restraint remain able to respond with a dramatic increase of serum corticosterone, various immune parameters, including spleen B- and T-lymphocyte proliferation, are unaffected (Durant et al. 1993; Fitzpatrick et al. 1992). However, in vivo, NOD mice appear to be resistant to thymus cell depletion caused by acute and chronic stress and/or infection, and in vitro, as mentioned above, NOD thymocytes are resistant to glucocorticoid-induced apoptosis (Casteels et al. 1998; Durant et al. 1993; Martins and Aguas 1999). Although up-regulated expression of the bcl-2 gene has been postulated, evidence suggests that the molecule responsible for this resistance acts up-stream from the bcl-2 gene (Garchon et al. 1994; Martins and Aguas 1996). It has also been shown that in NOD mice, the glucocorticoid response involves a prolonged exposure to high levels of the survival signal represented by the Myc protein, which might delay thymocyte and splenocyte apoptosis (Martins and Aguas 1998).

Adrenalectomy, which reduces corticosterone secretion (albeit not completely in mice), tends to accelerate diabetes onset, particularly in NOD males (Durant et al. 1993; Saravia-Fernandez et al. 1996a). The number of islet glucocorticoid receptors in all types of endocrine pancreatic cells decreases appreciably at 3 wk of age, but with no sexual dimorphism (Thompson et al. 2002). This glucocorticoid-receptor loss might contribute to the onset of insulitis and potentially to the ontogeny of diabetes in NOD mice as a result of loss of immunosuppressive glucocorticoid effects, particularly on cytokine production (Perretti et al. 1989). Pertinently, in prediabetic NOD islets, IL-1 receptors are expressed with the same density, affinity, and specificity as in control strains (Jafarian-Tehrani et al. 1995).

Researchers have also assessed the effect of melatonin, a hormone produced by the pineal gland and known to counteract glucocorticoid-induced thymus atrophy and immunosuppression in NOD females. Conti and Maestroni (1996) have reported that neonatal pinealectomy accelerates diabetes development, and exogenously administered melatonin protects the animals, in spite of its enhancement of insulin autoantibodies (IAAs1).

**Infections**

Infections generate opposite effects on T1D, as described above for other environmental factors. First, they have an apparent protective effect against both NOD and human T1D (Bach 1994). For example, in susceptible strains of mice or rats, autoimmune diseases, particularly T1D, develop earlier and more frequently among animals bred in a specific pathogen-free environment. Indeed, infecting young NOD mice with various pathogens can prevent diabetes (Bach 2002a). It is unknown to what extent this protection might be the partial result of long-term immunosuppression of glucocorticoids, whose secretion is triggered by cytokines (Bateman et al. 1989; Besedovsky and del Rey 1996; Fukata et al. 1994; Tilders et al. 1994).

Second, among environmental factors triggering T1D, much attention has been focused on viruses (Jun and Yoon 2003). To date, 13 different viruses have been reported to be associated with T1D development in humans and various animal models. The most clear and unequivocal evidence
that a virus induces diabetes in animals comes from studies on the encephalomyelitis virus in mice and the Kilham rat virus in rats (Jun and Yoon 2003). In human diabetes, several other viruses in addition to the enteroviruses have been considered potentially causal agents (Hyoty and Taylor 2002). With the exception of congenital rubella, their role is not clear. Enteroviral infections appear to accompany or precede the onset of diabetes in children. As with rubella, enteroviral infection may induce beta-cell autoimmunity even in utero (Dahlquist et al. 1999; Otonkoski et al. 2000). The following several mechanisms have been advanced to explain how viruses might trigger T1D: (1) Sequence similarities between islet-cell GAD and coxsackievirus could cause an immune attack against coxsackievirus and also beta cells. (2) Enteroviral infections could sustain autoimmunity until a final “hit” results in beta-cell loss. (3) Acute or chronic enteroviral infections in peri-insular tissue, because of excess production of free radicals, could cause beta-cell destruction (Graves et al. 1997; Jun and Yoon 2003). However, there is also evidence in humans that increased insulin resistance during infection (e.g., a flu-like viral infection) might cause insulin deficiency in individuals with widely fluctuating residual beta-cell activity (Wasmuth et al. 2000). Even moderate infections can be responsible for severe insulin resistance, with striking plasma insulin increases to maintain normal glycemia (Sammalkorpi 1989; Yki-Jarvinen et al. 1989). Pertinently, after a single injection of IL-1, an inflammatory cytokine, NOD females mount a huge corticosterone response, which induces a transient state of insulin resistance that prevents the IL-1-elicited hypoglycemia that occurs normally (Amrani et al. 1996). When mice are adrenalectomized before IL-1 injection, hypoglycemia characterizes only the NOD females, thus demonstrating the role of glucocorticoids in their IL-1-induced insulin resistance. NOD males, despite their higher insulin secretion and lower corticosterone response, compared with females, are resistant to the IL-1-induced hypoglycemic effect. Furthermore, their strong IL-1-induced insulin secretion is androgen dependent, because it is suppressed by orchidectomy (Amrani et al. 1996). In normal rats, testosterone has a direct effect on pancreatic islet function by favoring insulin gene expression and secretion (Moriyama and Eisenbarth 2002). Hence, androgens appear to be intricately involved in the IL-1-induced insulin resistance of NOD males. The androgen-induced insulin resistance has been described in other mouse strains (Leiter 1989).

**Ambient Temperature**

Low ambient temperature may be considered a stressful condition that alters glucose homeostasis (MacDonald et al. 1987). In this context, NOD mice placed at 23.7°C upon weaning have a lower diabetes incidence at 30 wk of age than those placed at 21.1°C, yet the degree of insulitis does not differ (Williams et al. 1990). Animals housed at the lower temperature have a transiently higher food intake between 13 and 15 wk of age that stimulates beta cells. Pertinently, children’s overeating has recently been cited as partly explaining the increasing incidence of juvenile T1D, as for type 2 diabetes (T2D) (Pundziute-Lycka et al. 2003). Cold temperature and overeating, and their potential relationship, should be investigated more thoroughly as factors favoring diabetes expression along the so-called T1D North-South gradient, just as cold temperature and infections might explain increased seasonal (autumn/winter) T1D incidence, perhaps concomitantly with overeating (Bach 2002b; Dahlquist et al. 1999). Moreover, a cold environment is also associated with low exposure to sunlight, which might lead to vitamin D deficiency (Bach 2002b). As mentioned above, vitamin D has been shown to protect against T1D.

**Proteins, Free Fatty Acids, and Carbohydrates**

Epidemiological and animal studies in BB rats and NOD mice have shown that diabetes is a food-influenced disease. Notably, the timing of dietary changes is important, because the maternal diet influences diabetes in NOD offspring (Funda et al. 1999; Reddy et al. 1995). Although the data concerning the diabetogenic effects of milk proteins have become very controversial, wheat flour and soybean proteins are still thought to be the major identified diabetogenic foods (Hoofar et al. 1993; Paxson et al. 1997; Scott and Marliss 1991; Scott et al. 1988). NOD mice fed a gluten-free diet, while maintaining the same potentially diabetogenic protein (e.g., soybean and milk proteins) consumption and never exposed to gluten even in utero because NOD breeding pairs ate same diet, evidence a much lower T1D incidence (Funda et al. 1999). The mechanisms by which the different diabetogenic components act on diabetes development in BB rats and NOD mice are still unknown, but the involvement of the mucosal immune system (e.g., in celiac disease), which is often associated with T1D, is not excluded.

Essential fatty acid deficiency (EFAD) has an antidiabetogenic effect in multiple low-dose streptozotocin-treated mice and BB rats (Leftkowith et al. 1990; Wright et al. 1991). NOD females fed an EFAD diet from weaning have a sharply decreased cumulative T1D incidence, but the insulitic process was unaffected (Benhamou et al. 1995). The results of studies on various immune parameters suggested a heightened activity of TH2-like cells, concomitantly with an effect on antigen-presenting cells (APCs), which is linked to less eicosanoid metabolism. Pertinently, we demonstrated that peritoneal MΦs of prediabetic NOD mice have enhanced production of prostaglandins and leukotrienes (Lety et al. 1992). A final important dietary component that influences diabetes is that of carbohydrates. As for some other environmental factors, the timing of exposure to glucose appears to be important. Although NOD mice rarely become diabetic during their first gestation (indeed, they are slightly hypoglycemic because of hyperinsulinemia; our unpublished ob-
were examined in NOD mice treated from 3 wk of age. Tolbutamide, which stimulates insulin secretion, increased glucose tolerance and insulin secretion, elevated IGF-1 levels, and preserved beta-cell function against developing diabetes when administered from weaning (Orban et al. 2001). NOD mice fed fructose have increased insulin-receptor substrate, and preserved beta-cell function (Nakajima et al. 1985). However, insulin and cumulative insulin resistance are lower than for the control group. Prophylactic insulin administration prevents insulin resistance and diabetes in NOD mice and BB rats, but available clinical trial data are not convincing (Pozzilli 2002). Both NOD and NODscid mice receiving this therapy develop fewer mega-islets (Jansen et al. 1996; Rosmalen et al. 2002a). Moreover, treated NODscid mice have less APC infiltration, indicating that the protective effect is not solely the induction of immunological tolerance to insulin, which is considered to be a major autoantigen (Karounos et al. 1997). These observations suggest one or more important links between the functional status of the islet, leukocyte infiltration, mega-islet formation in NOD and NODscid mice, and—in only in NOD mice—between insulin and diabetes (Rosmalen et al. 2002a).

Thiazolidinediones (TZDs) are drugs that act as receptor agonists to reduce insulin resistance. They are currently used in combination with other hypoglycemic agents to treat T2D, and their effects on NOD mice have been evaluated. TZDs are also of interest because they possess anti-inflammatory properties that may have the potential to limit immune-cell inflammation that occurs in the islets of T1D. Both rosiglitazone and troglitazone, given by gavage from 3 wk of age, lower diabetes incidence. However, troglitazone has marked hepatotoxicity at the dose used (Beales and Pozzilli 2002). By down-regulating IL-1β-induced intercellular molecule adhesion-1 expression on islet cells, troglitazone modifies the ability to target autoimmune beta-cell destruction. By modifying IFNγ production, it also acts on T cells, the major effectors of the autoimmune process (Augstein et al. 2003).

Insulin Growth Factors

IGF-1, which shares structural homology with insulin and has an insulin-like metabolic effect, has been given to NOD females (Kaino et al. 1996; Yakar et al. 2001). Only early short-term (4-9 wk of age) but not long-term (4-34 wk of age) treatment delays diabetes onset and limits insulin. IGF-1 also lowers the T1D incidence after transfer of autoimmune T cells from diabetic mice to nondiabetic NOD recipients (Bergerot et al. 1991). In these transfer experiments, IGF-1 treatment contains insulin and T-cell trafficking to lymphoid organs. Moreover, beta-cell mass, as assessed by insulin immunolabeling, is better preserved in IGF-1–treated mice than in controls, but pancreatic insulin contents and amounts of mRNA proinsulin transcripts are similar. Pertinently, IGF-1 is known to stimulate prolifera-

The effects of diazoxide, which inhibits insulin secretion, and tolbutamide, which stimulates insulin secretion, were examined in NOD mice treated from 3 wk of age (Williams et al. 1993). Only tolbutamide lowers their diabetes incidence, but high dose levels of tolbutamide aggravate insulitis. Monosodium glutamate administered to neonatal NOD females results in obesity associated with diabetes, hyperinsulinemia, and elevated corticosteronemia (Nakajima et al. 1985). However, insulin and cumulative insulin resistance are lower than for the control group. Prophylactic insulin administration prevents insulin resistance and diabetes in NOD mice and BB rats, but available clinical trial data are not convincing (Pozzilli 2002). Both NOD and NODscid mice receiving this therapy develop fewer mega-islets (Jansen et al. 1996; Rosmalen et al. 2002a). Moreover, treated NODscid mice have less APC infiltration, indicating that the protective effect is not solely the induction of immunological tolerance to insulin, which is considered to be a major autoantigen (Karounos et al. 1997). These observations suggest one or more important links between the functional status of the islet, leukocyte infiltration, mega-islet formation in NOD and NODscid mice, and—in only in NOD mice—between insulin and diabetes (Rosmalen et al. 2002a).

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IGF-2 might be implicated in biological functions relevant to autoimmune diabetes, including inhibition of stimulation of beta-cell proliferation and apoptosis (Christoforl et al. 1995; Halminen et al. 1996; Hill and Hogg 1991). That IGF-2 is produced by lymphocytes and expressed in the thymus might be important in the control of the autoimmune response (Geenen and Lefebvre 1998; Nyman and Pekonen 1993; Vafiadis et al. 1998). The defective thymus IGF-2 expression observed in BB rats led to the hypothesis that this defect might be detrimental to insulin tolerance and favor diabetes development (Geenen and Lefebvre 1998).

**Leptin**

Finally, the impact of leptin, the product of the *obese* gene, has also been examined in NOD mice. Initial evidence suggests that its main function is to regulate body weight, energy balance, and endocrine functions (Lönqvist and Schalling 1997). However, leptin also influences immune and inflammatory responses, particularly, exerting proinflammatory effects (Fantuzzi and Faggioni 2000; Howard et al. 1999; Lord et al. 1998). In NOD females, serum leptin levels, which are already higher than those of NOD males or control strain females at 6 wk of age, increase sharply soon after the onset of hyperglycemia (Matarese et al. 2002). Intraperitoneal injections of recombinant leptin, when administered early in life, accelerate the development of autoimmune diabetes only in NOD females, enhance immune-cell infiltration, and augment IFNγ production by T cells, suggesting that leptin favors proinflammatory T-cell responses.

Thus, T1D is very sensitive to numerous hormonal and environmental factors. For this reason, it is not surprising that the diabetes incidence can vary among NOD colonies worldwide (Pozzilli et al. 1993). Moreover, it is worth noting that in spontaneous animal models of T1D and in human disease, factors such as infections, dietary modifications, stressful events, puberty, and pregnancy, which increase insulin resistance and beta-cell activity thereby lowering resistance of T1D-prone individuals to microenvironmental factors involved during the progression of insulitis, could exacerbate the insidious progression to T1D. The effect of puberty, which intervenes immediately after weaning in rodents, has not been evoked until now, except indirectly in terms of castration in NOD mice. However, in humans, puberty, which induces insulin resistance, is well recognized as a factor triggering the clinical onset of both T1D and T2D (Amiel et al. 1991; Bergstrom et al. 1996; Caprio et al. 1989). One important feature of puberty is rapid growth, and growth hormone is known to increase peripheral insulin resistance (Amiel et al. 1986). Finally, in addition to its transmissible genetic origin, it has been suggested that human T1D diabetes is a congenital disorder (Bruining and Batstra 1999; Todd and Wicker 2001). Evidence related to human T1D has accumulated to indicate that the pathogenetic process may start early in life, even in utero, with increased risk observed (1) for maternal-child blood group incompatibility, which is associated with beta-cell dysfunction; (2) with older maternal age, excess weight gain during pregnancy, and amniocentesis (Vaarala et al. 1999); and, as mentioned above, (3) with some viral infections during pregnancy.

**Characteristics of NOD Glucose Homeostasis and Its Regulation During the Prediabetic Stage**

**Perinatal Pancreas Abnormalities in Autoimmune Diabetes**

Some of the data presented above would seem to indicate that the clinical expression of T1D is “programmed” as early as intrauterine life in NOD mice. Additional NOD and human T1D data presented below reinforce the hypothesis. NOD neonates born to mildly hyperglycemic mothers have greater birth weights than control newborns (Formby et al. 1987). Maternal metabolic anomalies lead to high glucose levels in utero, which stimulate fetal beta cells and increase birth weight, due to the pleiotropic effects of insulin. We also studied various parameters that affect the pancreas and glucose homeostasis of NOD and NODscid neonates born to young nondiabetic mothers (7 wk old at mating) and compared them with those of C57BL/6 newborns (Homo-Delarche et al. 2003). Pancreatic insulin contents and mean islet size, assessed by insulin immuno-labeling, are similar in the three strains during the first month of life. Antigliucagon labeling of NOD and NODscid pancreata reveals high percentages of small glucagon* islets that decline within the first 2 wk of age, and is suggestive of islet neogenesis (Pelegri et al. 2001). Concomitantly, compared with control C57BL/6 mice, both NOD strains are histologically characterized at birth by higher numbers of large and irregularly shaped islets that also progressively disappear (S. Geutkens, F. Homo-Delarche, J. M. Pelayo, S. Durand, H. A. Drexhage, and W. Savino, manuscript in preparation). Reverse transcriptase-polymerase chain reaction detected enhanced expression of preproinsulin I and II in both NOD and NODscid mice between 1 and 2 wk of life.

Notably, in all mouse strains investigated, including controls, pancreatic preproinsulin II expression is 10-fold higher than that of preproinsulin I. In situ hybridization revealed that 1-day-old NOD mice express significantly higher levels of primary preproinsulin II transcripts than age-matched C57BL/6 mice, but this difference disappeared during the first week of life. When we treated NOD and NODscid mothers with insulin (Ultratard, NovoNordisk, 1 U/100 g BW) during the last 2 wk of gestation, insulin transcriptional activity at birth was down-regulated in female NOD mice but not in males or both sexes of NODscid. These findings demonstrate that signs of beta-cell stimulation exist in NOD and NODscid neonates. We cannot exclude that insulin resistance, possibly linked to HPA-axis disturbances, might play a role in both NOD and NODscid
mice (our preliminary results). Moreover, in newborn NOD mice, sexual dimorphism is already manifest, reflected in a lack of pancreas preproinsulin II down-regulation in males after maternal prophylactic insulin administration. Such a lack is perhaps due to insulin resistance induced by androgens, which are known to increase during the few days before birth in males, exclusively (Weisz and Ward 1980).

In humans, some evidence supports a weak, albeit significant, association of higher birth weight and increased risk for T1D, with the possibility of sexual dimorphism (Podar et al. 1999; Stene et al. 2001). Moreover, results of a retrospective study have indicated that children who later developed T1D gained more weight (body mass index) during the first year of life than their healthy siblings (Bruining and Batstra 1999). Taken together, these data suggest that perinatal beta-cell hyperactivity is associated with a higher risk of developing diabetes.

Postweaning Anomalies of the Pancreas and Glucose Homeostasis in T1D

The accumulation of DCs and MΦs, particularly at the duct and islet periphery, which is generally thought to be the first sign of the autoimmune process in NOD mice and BB rats, usually starts soon after weaning (Figure 2). We investigated glucose homeostasis in prediabetic mice because of reported heightened beta-cell sensitivity to glucose observed in perfused inflamed islets from prediabetic BB rats compared with noninflamed islets and transient hyperinsulinemia accompanying insulin resistance during the prediabetic phase of T2D (Figure 3) (DeFronzo 1997; Teruya et al. 1993). We detected transient hyperinsulinemia in both sexes of NOD and NODscid mice compared with several control strains (C57BL/6, BALB/c, DBA/2), indicating that their beta cells are hyperactive during the prediabetic period (Amrani et al. 1998; Homo-Delarche 1997). Others have confirmed the beta-cell hyperactivity in NOD, compared with BALB/c mice (Orban et al. 2001). This mild hyperinsulinemia develops between 2 and 4 wk of age, or about the time of weaning, when the shift from maternal milk to laboratory chow takes place. Even in normal mouse strains, the sharply increased dietary glucose at this time awakens beta cells, which have been at rest because of the low glucose content of maternal milk (Girard et al. 1992). At 8 wk of age, basal nonfasting blood insulin levels are still higher in NOD and NODscid mice than in controls (Amrani et al. 1998). Mild hyperinsulinemia in NOD and NODscid mice is associated with decreased glycemia, as expected, compared with sex-matched C57BL/6, DBA/2 and BALB/c mice (Homo-Delarche 1997). Because glucose has been shown to protect beta cells, this low glycemia level might lower their resistance to the various inflammatory factors produced in the islet microenvironment (Hoorens et al. 1996). It is pertinent that transiently elevated insulinemia has also been observed in the BB rat a few days before the onset of T1D, and, as mentioned above, perfused inflamed, but not uninflamed, islets exhibit beta-cell hyperactivity (Nakhooda et al. 1978; Teruya et al. 1993). A defect in the

Potential Beta-cell Stimuli During the Early, Relatively Long, Prediabetic Period in Autoimmune Diabetes

High in utero glucose levels in NOD mice can result in relatively greater insulin secretion at weaning, because of the existence of the so-called “glucose-priming effect” (Formby et al. 1987; Fujimoto et al. 2000). This effect predicts that the more beta cells have previously been stimulated, the higher their insulin response to a rise in glucose levels will be. Thus, postweaning islet abnormalities might be the consequence of earlier altered beta-cell activity that spontaneously normalizes after birth but reappears at weaning together with beta-cell awakening induced by diet change and islet microenvironment linked to the onset of the inflammatory reaction. However, signs of beta-cell stimulation are seen in NOD pups, whose young mothers (7 wk of age at mating) were not diabetic before and after gestation.

Intriguingly, as described above, signs of molecular beta-cell stimulation are also observed in lymphocyte-deficient NODscid neonates, whose mothers never become diabetic. Pertinently, NODscid mice have an unexpected pattern of higher-level basal glycemia before weaning than C57BL/6 and NOD mice, with higher insulinemia but low glucagonemia (Pelegri et al. 2001). To our knowledge, it is presently unknown whether insulin resistance exists in mice with the scid mutation. However, athymic BALB/c nude mice develop spontaneous hyperglycemia, impaired glucose tolerance and insulin insensitivity early in life, which makes them a good model of T2D (Zeidler et al. 1991, 1992). They also exhibit HPA-axis disturbances at the hypothalamus level (Spinetti et al. 1997). On the basis of these data and the fact that the NOD background derives from a common ICR progenitor shared with the nonobese nondiabetic (NON(NOD)) strain, a model of pre-T2D, some insulin resistance on the NOD background might indeed occur (Atkinson and Leiter 1999). Moreover, with regard to glucose homeostasis, NODscid mice might share some similarities with nude mice, perhaps related to HPA-axis perturbations. We have evidence of HPA-axis hypersensitivity in NOD and NODscid mice during gestation and of the abnormal response of this axis to IL-1 in NOD pups before weaning (unpublished results).

With regard to postweaning hyperinsulinemia, other factors (e.g., infiltrating cells and their cytokine products) might also be involved, either affecting the endocrine cells of the islet directly or altering their innervation. As to whether such factors act directly on beta cells, because low concentrations of cytokines (e.g., IL-1) stimulate insulin secretion in vivo and in vitro, inflammatory cells might indeed contribute to beta-cell hyperactivity (Wogensen et al. 1990; Zumsteg et al. 1993). Pertinently, we have shown that DCs from young adult prediabetic NOD mice stimulate in vitro insulin secretion by NOD prediabetic islets (Durant et al. 2002). Mild hyperglucagonemia has been observed simultaneously with hyperinsulinemia in NOD and NODscid mice (Ohneda et al. 1984; Pelegri et al. 2001). The causes of this hyperglucagonemia could again be linked to cytokines (e.g., IL-1), which also stimulate alpha cells that also appear to contain IL-1 receptors (Jafarian-Tehrani et al. 1995; Wogensen et al. 1990; Zumsteg et al. 1993).

Another nonmutually exclusive explanation for hyper-
glucagonemia might be defective regulation of glucagon secretion, which is normally tightly controlled by the ɣ-aminobutyric acid (GABAergic) glutamic acid decarboxylase (GAD1)-containing innervation (Sorensen et al. 1991). In NOD and control mouse pancreata, as in rats, neurons and beta cells express GAD (Saravia-Fernandez et al. 1996b; Sorensen et al. 1991). Moreover, lymphocytes infiltrate around GABAergic fibers in 1-mo-old NOD pancreata (Saravia-Fernandez et al. 1996b). The fibers then disappear rapidly in NOD females but persist in control mice. After weaning, when transient beta-cell hyperactivity starts, autoantibodies to peripherin, a marker of developing innervation, and T-cell reactivities against peripherin and GAD67 appear in NOD mice (Boitard et al. 1992; Kaufman et al. 1993; Saravia and Homo-Delarche 2003; Tisch et al. 1993). Schwann cells (Scs), which accompany axons, envelop the islet at its periphery, as nerves do, as if delimiting the islet and exocrine parenchyma (Honjin 1956; Saravia and Homo-Delarche 2003; Winer et al. 2003). Pertinently, in 1-mo-old NOD females, T cells are attracted to the endocrine-exocrine junction, where they accumulate (Winer et al. 2003). T cells then penetrate into the Sc mantle and usually concentrate contiguous to it, rather than moving into the central islet beta cells. Scs progressively disappear, before beta-cell deficiency appears, and 1-mo-old NOD females already have autoreactive T cells and autoantibodies specific to glial fibrillary acidic protein (GFAP1). In contrast, Scs are not destroyed in control mice, NODscid, or after chemically induced beta-cell damage (Winer et al. 2003). Injection of GFAP-specific T-cell lines into NODscid mice alters the Sc envelope but does not induce beta-cell destruction and diabetes. Therefore, peri-islet nerves might be a precocious actor in TID pathogenesis, as we recently suggested on the basis of their resemblance to beta cells (common molecule expression and cellular processes (Saravia and Homo-Delarche 2003). On a genetic basis, polymorphisms in the NeuroD/BETA2 gene may be linked to TID (Hansen et al. 2000; Iwata et al. 1999; Owerbach et al. 1997). NeuroD/BETA2 is essential in the mouse not only for beta-cell morphogenesis and differentiation, but also for neurons (Naya et al. 1997).

Gene-determined Alterations of Pancreatic Hormone Expression at the Pancreas and Thymus Levels in Autoimmune Diabetes

Specific genes or alleles unique to diabetes have not been identified in either human or NOD mouse mice. Instead, the risk of disease development is determined by an unfortunate combination of permissive alleles that, by themselves, are also found in the genomes of nondiabetes-prone individuals and that culminate in diabetes only when combined with specific environmental factors (Pugliese and Miceli 2002).

To date, genetic studies in NOD mice have mainly identified chromosomal loci associated with immune system abnormalities, and the pancreas has received little attention (Todd and Wicker 2001). In human patients with TID, there are also indications of a genetic basis underlying aberrant islet function. Among the TID susceptibility loci, the IDDM2 locus maps to a variable number of tandem repeats (VNTR), which are minisatellites of the insulin gene. The short class I VNTR alleles predispose to TID, whereas the long class III VNTR alleles have a protective effect (Pugliese and Miceli 2002; Todd and Wicker 2001). Predisposing class I VNTR alleles are associated with higher pancreatic but lower thymus preproinsulin-mRNA levels than protective class III VNTR alleles, which are therefore associated with low pancreatic preproinsulin expression. By contrast, human T2D is associated with the class III VNTR (Huxtable et al. 2000; Ong et al. 1999) and low pancreatic mRNA preproinsulin expression (Bennett et al. 1995; Vafiadis et al. 1996). In human T1D, it is unknown whether these different levels of expression in thymus (via tolerance induction) and/or in pancreas (via sensitivity to autoimmunity) are responsible for the difference in diabetes susceptibility. Nevertheless, specific polymorphisms in the insulin gene region do appear to be significantly more frequent in concordant monozygotic twins than discordant twins (She and Marron 1998).

Epidemiological studies have revealed that the TID incidence is lower for children of diabetic mothers than for those with diabetic fathers (Todd and Wicker 2001). These observations may be explained by imprinting, a mechanism that regulates gene expression by silencing either the maternal or the paternal allele (Pugliese and Miceli 2002). The IGF2 gene, which is adjacent to the insulin gene, was the first human gene found to be imprinted, and it is expressed exclusively on the paternal chromosome (Polychronakos et al. 1995). Hence, imprinting and parent-of-origin effects appear to be important modulators of insulin gene (INS) expression and, in turn, susceptibility to both T1D and T2D (Pugliese and Miceli 2002). Finally, as for NOD mice, epidemiological studies have shown that parental T2D increases the risk for T1D in their offspring, suggesting that T1D and T2D thereby share some common genetic factors (Kajo et al. 1995; Rich et al. 1991).

In NOD mice, attempts have been made to assess the role of thymic expression of potential pancreatic autoantigens, particularly insulin. The thymus, as a primary lymphoid organ, promotes the proliferation and differentiation of a specific T-cell repertoire through which the immune system distinguishes “self” antigens from invading pathogens and mutagens (Mondino et al. 1996; Rosmalen et al. 2002b). The thymus produces various cytokines and humoral factors, which influence thymocyte differentiation in an autocrine and/or paracrine manner, as well as neuroendocrine and peripheral hormones, which also modulate thymus physiology (Savino and Dardenne 1995). It is thought that intrathymic presentation of neuroendocrine self-antigens by or in close association with major histocompatibility complex (MHC1) proteins is responsible for the establishment of central immune tolerance of neuroendo-
crine molecules (Geenen and Brilot 2003). For this reason, attention has been focused on thymic expression of various factors, some of which have been considered to act as autoantigens, particularly in T1D (Geenen and Lefebvre 1998; Throsby et al. 1998).

We and others have described the expression in the mouse thymus of (1) pancreatic endocrine hormones such as preproinsulin II, which is considered together with insulin major potential T1D autoantigens, proglucagon, proinsomatostatin, and propancreatic polypeptide; (2) exocrine hormones, such as amylase, elastase, and trypsin; and (3) an isoform of GAD67, which is considered to be a major potential autoantigen in T1D (Chentoufi and Polychronakos 2002; Jolicoeur et al. 1994; Throsby et al. 1998; Throsby et al. 1999). Moreover, all members of the insulin gene family are expressed in the thymic stroma according to the following precise hierarchy and cell topography:

\[ \text{IGF-2 (thymic epithelial cells)} > \text{IGF-1 (thymic Mφ)} > \text{insulin (thymic epithelial cells and/or DC)} \] (Geenen and Brilot 2003). Mφs were reported to express proglucagon, proinsomatostatin, and propancreatic polypeptide (Throsby et al. 1998).

Thus, investigators have attempted to correlate autoantigen expression levels with induction and/or maintenance of tolerance in humans, rats, or transgenic mice (Adams et al. 1987; Heath et al. 1998; Pugliese et al. 1997; Smith et al. 1997; Vafiadis et al. 1997). However, until fine quantification at the level of the various, well-defined cell types susceptible to express these hormones becomes available, comparison will be difficult (Pleau et al. 2001).

Unlike humans, lower mammals show no VNTR upstream from the gene encoding insulin. However, rodents express two proinsulin isoforms, encoded by distinct genes that are located on chromosomes 7 and 19 in the mouse. The two isoforms differ by two amino acids in the B-chain, three amino acids in C-peptide, and several differences in the leader peptide sequence (Dubois-Lafforgue et al. 2002; Philippe 1991). One isoform, proinsulin I, is expressed exclusively in islets. The second isoform, proinsulin II, is abundantly expressed in islets and also, at very low levels, in selected other tissues, particularly the thymus. In adult rodent pancreas, proinsulin II is more abundantly expressed than proinsulin I, whereas an inverse ratio is observed during organogenesis (Deltour et al. 1993). T cells specific to four proinsulin epitopes were identified in the mononuclear cell infiltrate of prediabetic NOD females (Halbout et al. 2002). Immunogenic epitopes are found on both isoforms and particularly proinsulin II, which is the isoform expressed in the thymus.

To elucidate the role of proinsulin in the development of T1D, NOD transgenic mice were generated for the mouse proinsulin II gene driven by an MHC class II promoter to direct expression of the transgene to MHC class II-bearing cells, including those of the thymus (French et al. 1997). Insulitis is almost completely absent and diabetes is prevented in these transgenic mice. Salivary gland infiltration (sialitis) and immune response to ovalbumin are not altered, indicating that the protective effect of the transgene is specific to islet pathology and not due to general immunosuppression. Inversely, homozygote proinsulin II−/− NOD mice develop accelerated insulitis and diabetes (Thebault-Baumont et al. 2003). These mice were characterized by the absence of preproinsulin II-mRNA expression but the presence of preproinsulin I (normally present in the pancreas but not in thymus) in both glands (Dubois-Lafforgue et al. 2002). Although IAAs are increased in proinsulin II NOD−/− mice, anti-GAD autoantibodies and sialitis remain unchanged (Moriyama et al. 2003; Thebault-Baumont et al. 2003). The detection of IAAs in proinsulin II−/− NOD mice confirms that proinsulin I is also an autoantigen in this model. Moreover, that study provided evidence that proinsulin II expression silences T cells specific to an epitope shared by proinsulin I and II (Thebault-Baumont et al. 2003).

These data on proinsulin II−/− NOD mice have been confirmed by others, who showed that by contrast, insulitis and diabetes are much lower in heterozygote proinsulin I−/− NOD mice and completely absent in most homozygote proinsulin I−/− mice (Moriyama et al. 2003). In that case, proinsulin II is expected to be expressed in both pancreas and thymus, but it is unknown to what extent. Despite the absence of insulitis, proinsulin II−/− NOD mice express similar levels of IAAs than proinsulin I−/− and wild-type NOD mice. Moreover, like the wild-type, proinsulin I−/− and proinsulin I−/− mice are not protected against sialitis. Finally, insulin I is not the only lymphocytic target of NOD mice, because proinsulin I−/− NOD islets, when transplanted into recently diabetic wild-type NOD mice, become infiltrated and only transiently reverse diabetes. These observations indicate that (1) loss of either insulin gene influences progression to diabetes; (2) thymic preproinsulin II expression silences T cells specific to an epitope common to proinsulin I and II, and (3) islets can be destroyed by lymphocytes targeting multiple molecules with evidence of epitope spreading.

The immune events thought to take place in the thymus during tolerance induction, their repercussions on the peripheral immune system, and their potential modulation by environmental factors are complex (Todd and Wicker 2001). Taken together, these data at the thymus level and those described above at the islet level underline the complexity of the alterations of the neuroendocrine-immune circuit that might lead to T1D.

Hypothetical Scenario of the Diabetogenic Process in NOD Mice

Endocrine/neuroendocrine and immune events in the order of their appearance at the NOD islet level and culminating in T1D are shown in Figure 4. Weaning, when there is a dietary change, is characterized by APC infiltration and the
onset of hyperinsulinemia, concomitantly with the decline of islet glucocorticoid receptors, which reduces the anti-inflammatory effects of glucocorticoids, as described above. On an immunological basis, weaning is considered to be the first "checkpoint" in NOD T1D, where peri-insulitis begins and thereafter progresses to severe insulitis (Andre et al. 1996)). The second checkpoint occurs at the onset of clinical disease, which corresponds to the change of the autoimmune status from "benign" to "malignant," manifested by destructive insulitis and loss of insulin secretion (Andre et al. 1996; Wegmann 1996). This second checkpoint might also have an endocrine basis, due to the diminishing levels of insulin, also known for its anti-inflammatory effects and, concomitantly, the proinflammatory effects of higher glucose and leptin levels (Dandona et al. 2003; Matarese et al. 2002). A neuroendocrine-immune approach to T1D pathogenesis might explain the different disease evolutions observed between NOD and T-cell receptor-transgenic NOD mice, in which both checkpoints also exist but are more clearly delineated.

Some puzzling data raise doubts concerning the currently acknowledged scheme of T1D pathogenesis, particularly that beta cells are considered the only targets in the context of, successively, MHC-class II and I interactions with CD4+ and CD8+ lymphocytes, respectively. First, as mentioned above, GFAP+ Scs and neurons surrounding the islet of Langerhans are destroyed before beta cells and antibodies and T-cell reactivities against the former appear early, about the time of weaning (Kaufman et al. 1993; Saravia and Homo-Delarche 2003; Tisch et al. 1993; Winer et al. 2003). Second, the interaction of beta-cell class I with MHC class I-restricted CD8+ T cells is not necessary for the initiation or early progression of insulitis, but it is important for the late switch from benign to malignant insulitis, suggesting that beta-cell MHC class I might be a late "requirement" for diabetes. Third, diabetes is accelerated in neonatal NOD mice transgenic for rat insulin promoter-tumor necrosis factor (TNFα), independent of the NOD allele at idd1 and the expression of functional MHC class II (Rajagopalan et al. 2003). These observations suggest that TNF expression in the pancreas might bypass the requirement of class II molecules for antigen presentation, and might lead to a direct effector pathway in the context of class I and CD8+ cells, independent of CD4+ cells, unless this TNF expression is deleterious for beta-cell viability. In light of these recent findings, one might ask whether it is possible to envision a coherent pathogenetic model of NOD T1D that intercalates all of the different target cells (e.g., Scs, neurons, and beta cells) in a logical progression.

We recently proposed a hypothetical scenario that attempts to solve the "murder mysteries" of autoimmune diabetes in NOD mice (Figure 5) (Kaufman et al. 1993; Saravia and Homo-Delarche 2003). Physiologically, perinatal neuronal loss occurs, especially in the sympathetic nervous system. The loss explains the presence of Fas+ nerves, which are observed perinatally in pancreata of all mouse strains (Durant et al. 2003). Sympathetic innervation, which is GAD+, is known to inhibit insulin secretion (Saravia and Homo-Delarche 2003; Sorenson et al. 1991). One can therefore envision that the NOD and NODscid beta-cell hyperactivity observed at birth might stimulate the fetal sympathetic innervation to down-regulate insulin secretion. After birth, as in control mice, this innervation will regress by apoptosis and will be eliminated by Mφs, because of the dietary change that induces beta-cell rest (Girard et al. 1992; Homo-Delarche et al. 2003; Saikumar et al. 1999). Normally, apoptosis does not trigger an inflammatory response, but in certain circumstances, apoptotic cells induce an im-

![Figure 4](https://https://academic.oup.com/ilarjournal/article-abstract/45/3/237/703443/16/March/2019)
A mune reaction that can increase susceptibility to or development of autoimmunity (Fadok et al. 2001).

In the context described above, NOD mouse M/M/H9021s present several defects in T1D—particularly defective phagocytosis of apoptotic beta cells, which could induce an inflammatory reaction adjacent to neurons and Scs, in turn stimulating cytokine production (IL-1, IL-6, TNF) (Durant et al. 2003; O’Brien et al. 2002). Damaged neurons and Scs are abnormally phagocytized by M/M/H9021s, and processed antigen- and MHC class II-expressing APCs would activate T cells in peripancreatic lymph nodes. At this point, the scenario rejoins that of Kaufman and colleagues (Kaufman et al. 1993; Saravia and Homo-Delarche 2003): Once activated, lymphocytes migrate to and accumulate around the islet periphery (peri-insulitis). T cells synthesize cytokines, particularly IFNγ, and propagate the autoimmune reaction that intensifies GAD-containing neuron and Sc destruction before that of endocrine cells. Breaches then form in the islet basement membrane, allowing lymphocyte infiltration (insulitis) and beta-cell targeting. Hyperactive endocrine cells are more susceptible to autoimmune reactions because of higher expression of autoantigens and adhesion and MHC molecules, and also because of their greater sensitivity to cytokine-induced damage (Homo-Delarche and Boitard 1996; Rosmalen et al. 2002a).

In Figure 6, the last part of the scenario that we advanced for the progression of the diabetogenic process and in which beta-cell hyperactivity plays a major role is illustrated. NOD beta-cell hyperactivity reaches its maximum between 6 and 8 wk (Amrani et al. 1998). Because beta cells have a limited life span, their hyperactivity in NOD mice should be accompanied by beta-cell renewal (Finegood et al. 1995). As a consequence, structurally normal islets of 4-wk-old NOD mice contain more somatostatin-pancreatic duodenal homeobox gene 1 (SOM+/PDX-1+) endocrine precursor cells than control ICR mice, and their number increases with age concomitantly with that of APCs (Fernandes et al. 1997). Evidence for ductal cell proliferation that is characteristic of neogenesis in 6-wk-old NOD mice has also been reported (O’Reilly et al. 1997). IAAs peak sharply in NOD mice 8 to 16 wk old (Yu et al. 2000). Moreover, IAA positivity at 8 wk of age (when beta-cell hyperactivity reaches its maximum) is strongly associated with early diabetes development in NOD mice but, curiously, not in the insulitis-resistant, diabetes-free nonobese resistant [NOR1] strain, which shares 85% of its genome with NOD mice, and in which a transient IAA peak is also observed (Abiru et al. 2001).

Beta-cell hyperactivity also leads to secretion of a potential autoantigen, proinsulin, which is normally not secreted (Rosmalen et al. 2002a). From 10 wk of age onward, NOD mice have a high percentage of mega-islets, which are specifically associated with infiltrating immune cells, and concomitantly enhanced beta-cell proliferation, suggesting
a compensatory proliferative response to ongoing autoimmune beta-cell destruction (Rosmalen et al. 2002a). Finally, beta-cell damage and subsequent decreased beta-cell mass induced by leukocyte infiltration lead to greater demands for insulin from the remaining beta cells, which also become hyperactive. Thus, a vicious cycle begins and ultimately results in the development of clinical symptoms of diabetes.

In Figure 7, the hypothetical successive steps of NOD beta-cell functional activity from the perinatal period to diabetes onset are briefly summarized. The potential causes underlying these different phases of beta-cell activity are indicated. In addition, insulin resistance from fetal life through adulthood might fluctuate, particularly if the mother is diabetic (Homo-Delarche 1997). Finally, this neuroendocrine-immune interpretation is able to incorporate environmental events known to influence T1D expression because most of them induce insulin resistance and compensating beta-cell hyperactivity.

Concluding Remarks

Despite their highly inbred characteristic, which therefore inadequately reflects the complexity of the human disease, the use of NOD mice makes it possible for scientists to study spontaneous evolution of T1D. The use of spontaneous animal T1D models allows sequential in situ studies at the pancreas level. Obviously, such studies are impossible in prediabetic patients.

Notably, one of the problems encountered in studying animal models is the choice of “control” strains. The most widely used controls (e.g., NOR or NODscid mice) are closely related strains. However, closely related strains might be inappropriate controls, inasmuch as several of the diabetes-predisposing factors could be missed because they are also potentially present in them. More precisely, the NOD strain shares a common origin with the NON strain, a pre-T2D model that offers the possibility of some common features, such as insulin resistance. Nevertheless, NODscid mice, which share several endocrine anomalies with NOD mice although to a lesser degree, are highly informative because their data suggest that these abnormalities are not triggered by lymphocytes.

In humans, although genetic risk markers appear to be necessary, they alone are not sufficient for the development of T1D, and the interactions of different genes with different environmental risk factors may be required (Dahlquist et al. 1999). Clearly, this situation holds true for the NOD mouse,
and elucidation of how its glucose homeostasis is regulated has improved our understanding of the possible mechanisms by which some of these environmental factors might be involved (Leslie et al. 1997). Indeed, we demonstrated a transient period of hyperinsulinemia in NOD and NODscid mice. These mice, particularly NODscid, exhibit mild insulin resistance, at least early in life. It should be noted that this sequence of events appears to exist also in prediabetic T1D patients, as recently reported (Fourlanos et al. 2003). Based on these endocrinological observations, T1D might be considered an "accelerated" form of T2D, whose evolution is complicated by defective cells of the innate and acquired immune systems on a T1D background, probably associated with genetic predisposition at the HLA or MHC in humans and rodents, respectively (Beyan et al. 2003; Delovitch and Singh 1997). This perspective might lead to a better integration of LADA patients, who present insulin resistance and slow autoimmune islet aggression. Furthermore, inflammation is increasingly thought to be linked to the pathogenesis of T2D and its complications (Dandona et al. 2003; Fernandez-Real and Ricart 1999; Pickup et al. 1997).

As recently suggested, T1D might be a disease linked to abnormal pancreas development, particularly its innervation, which is in close contact with islets and regulates their hormonal secretion (Durant et al. 2003; Homo-Delarche 2001; Saravia and Homo-Delarche 2003; Saravia-Fernandez et al. 1996b; Winer et al. 2003). Both T1D and T2D are modulated by environmental factors during prenatal life (Barker 1998; Dahlquist et al. 1999; Hattersley and Tooke 1999). One way for these events to intervene during the prenatal period is glucocorticoid "programming" (Clark 1998; Seckl 2001). In rodents and other species, antenatal exposure to glucocorticoids causes low birth-weight offspring and produces permanent hypertension, hyperglycemia, hyperinsulinemia, altered behavior, and neuroendocrine responses throughout their lives. A priori, these findings would appear to be more closely linked to T2D pathogenesis. However, glucocorticoid programming might also be considered in T1D pathogenesis. Indeed, processes underlying fetal programming include determination of the "set point" of the HPA axis and glucocorticoid expression (Seckl 2001). As mentioned above, we have obtained evidence, in gestating NOD mothers and at least in their female newborns, of early HPA-axis abnormalities evidenced by hyperactivity to various stressors. Such hyperactivity might down-regulate glucocorticoid receptors in some organs, as documented in the NOD pancreas as early as 3 wk of age (e.g., when the first immune cells, particularly MΦs and DCs, accumulate around ducts and islets). It is therefore possible that HPA-axis hyperactivity, regardless of its origin during fetal or perinatal life, might later diminish the natural anti-inflammatory and immunosuppressive effects of glucocorticoids. In the NOD T1D model, this effect begins about the time of weaning, when diet changes and pancreas growth and islet neogenesis can still be observed.

The numerous MΦ defects that exist in NOD mice, and particularly defective phagocytosis, might also trigger an inflammatory response in the early postnatal developing pancreas (Durant et al. 2003; Saravia and Homo-Delarche 2003). Indeed, it has been shown that both defective control of apoptosis and inflammatory signals can lead to organ autoimmunity (Saikumar et al. 1999; Vezys and Lefrancois 2002). Diminished glucocorticoid efficacy might further enhance the local MΦ-induced inflammatory response. ACTH levels in NOD mice decrease with age, although no corresponding change is detected in circulating corticosterone but adrenalitis is seen (Beales et al. 2002). Finally, this

Figure 7 Evolution of the beta-cell compartment, from fetal life to overt type 1 diabetes, and potential causes of each stage in the nonobese diabetic mouse as a function of age.
proinflammatory-prone profile will evolve toward autoimmunity because of additional defects of other APCs such as dendritic cells and lymphocytes (Delovitch and Singh 1997; Lam-Tse et al. 2002).

To what extent this NOD polyclonendocrine disease characterized by insulitis, adrenalitis, sialitis, parathyroiditis, and thyroiditis might be linked to defective glucocorticoid activity deficiency remains to be determined. Notably, several other animal and human autoimmune diseases are associated with HPA-axis disturbances and diminished glucocorticoid efficacy. Examples include obese strain chickens with spontaneous thyroiditis; murine models of lupus; and the BB rat, whose corticosteroid-binding globulin (CBG\(^1\)) contains an amino acid substitution and has lower affinity for glucocorticoids than Wistar CBG (Bese-dovsky and del Rey 1996; Smith and Hammond 1991). Such a CBG disturbance, already present during fetal life, results in less effective glucocorticoids and might be responsible for the neonatal mortality of and frequent respiratory infections in BB rats (Like et al. 1982). It is worth noting that bipolar (manic depressive) patients have a high prevalence of autoimmune thyroiditis (Lam-Tse et al. 2002). They also have HPA-axis disorders responsible for hypercortisolism and glucocorticoid insensitivity. Pertinently, this glucocorticoid insensitivity exists at the T-cell level in these patients and favors development of autoimmunity (Lam-Tse et al. 2002). Taken together, these observations suggest that defective anti-inflammatory effects of glucocorticoids, whether they are consequences of environmental, prenatal, or perinatal factors, should be considered in both T1D and T2D.

In conclusion, the NOD mouse, although not duplicating all of the clinical features of human T1D, appears nevertheless to be a good model for the understanding and analysis of the complex neuroendocrine-immune interactions that underlie the onset and progression of T1D and potential relationships with environmental factors. Primarily, the model has made it possible for scientists to elucidate events—particularly those concerning islet innervation—that occur during the perinatal period and the early transient stages of beta-cell hyperactivity (Rosmalen et al. 2002a; Saravia and Homo-Delarche 2003). Information obtained with the NOD model prompts us to wonder whether a given autoantigen is required to initiate autoimmune diabetes. Rather, as emphasized elsewhere, “it remains possible that a still unrecognized combination of genes and environmental factors triggers the destruction of beta cells and that the autoimmune response is secondary to this process” (Notkins and Lernmark 2001, p. 1251). That possibility might explain the plethora of autoantigens present in T1D and facilitate the understanding of the immune mechanisms.

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