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The postnatal maternal environment influences diabetes development in nonobese diabetic mice

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Abstract

When nonobese-diabetic (NOD) mouse embryos were implanted into pseudopregnant mothers of a nonautoimmune mouse strain, the progeny had a reduced type 1 diabetes (T1D) incidence, suggesting that transmission of maternal autoantibodies is important for T1D development. Whether eliminating islet autoantibody transmission *in utero*, or postnatally (through milk), prevented T1D is unknown. Herein, we show that fostering newborn NOD mice on B-cell deficient NOD.Igμ^{-/-} dams does not prevent T1D, demonstrating that postnatally transmitted islet autoantibodies are not required for disease pathogenesis. Additionally, NOD.Igμ^{-/-} mice reared on NOD dams did not develop T1D, indicating that autoantibody transmission to B-cell deficient NOD neonates is insufficient to trigger T1D. Interestingly, newborn NOD mice that were reared by ICR (but not NOD or C57BL/6) dams had reduced T1D incidence, although not as reduced as that reported after embryo transfer to ICR mice, suggesting that both prenatal and postnatal factors contribute to the observed reduction in T1D incidence. Thus, NOD mice have different risks for developing T1D depending on the strain of their foster mother, and both prenatal and postnatal maternal factors, other than islet autoantibodies, influence their T1D incidence. The results may be relevant for understanding the increasing incidence of T1D and designing interventions.

Keywords

autoantibodies; maternal factors; NOD mouse; type 1 diabetes; milk

Introduction

Type I diabetes (T1D) results from insulin deficiency due to the autoimmune-mediated destruction of the insulin-producing β-cells. It arises from an incompletely understood interaction between β-cells, the immune system and the environment in genetically susceptible individuals [1,2]. Autoantibodies to β-cell antigens appear years before disease onset and remain detectable long after disease onset. Since the placenta expresses FcRn receptors that transport IgG maternal antibodies across the placenta [3], infants of mothers with T1D are often born with autoantibodies to insulin, glutamic acid decarboxylase, tyrosine phosphatase IA-2 and other βcell antigens [4–7]. Postnatally, maternal IgG antibodies in breast milk are transported across the intestinal epithelium to the neonatal circulation, which is an important

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mechanism for protecting newborns from pathogens until their immune system matures [8–11].

Two studies recently reported that maternally transmitted autoantibodies may affect NOD mouse progeny's risk for developing T1D. Greeley et al. implanted NOD embryos into three types of non-autoimmune pseudopregnant mice; 1) NOD.Igμ^{-/-} mothers (which are B cell-deficient), 2) immunoglobulin transgenic NOD mothers which make antibodies only against hen egg lysozyme (α-HEL NOD mice), or 3) wildtype DBA mothers [12]. Approximately 15–25% of the female NOD mice arising from all three non-autoimmune mouse strains had T1D at the end of the observation period (to 40 weeks of age), compared to about 60–70% of the controls. These observations led those investigators to suggest that maternal transmission of autoantibodies is a critical environmental factor influencing the risk for developing T1D. Kagohashi et al. also observed that NOD mice produced from embryo transfer into ICR and DBA mothers had a very low incidence of T1D, with only about 7% of the mice developing T1D by 40 weeks in age [13]. Interestingly, although these mice were protected from T1D, they developed insulinitis significantly earlier than usual. These studies did not distinguish whether it was the elimination of autoantibody transmission *in utero*, postnatally, or both, that reduced disease incidence in the NOD progeny.

Therefore, we tested the extent to which elimination of islet autoantibody transmission postnatally through milk would effect T1D development in NOD mice. Our observations do not support the notion that transmission of islet autoantibodies through mother's milk represents a risk factor for T1D. In addition, we show that adopted NOD mice are differentially protected from T1D depending on the strain of their foster mother. When protection from T1D was observed, it was not as great as that reported after transfer of NOD embryos to that mouse strain, suggesting that prenatal factors can also modulate risk for T1D. We discuss the other factors in the prenatal and postnatal maternal environment that may modulate the T1D incidence of adopted NOD mice.

Methods

Mice

In our NOD mouse colony (originating from Taconic Farms) approximately 80% of female mice develop T1D by 35 weeks of age. Institute of Cancer Research (ICR) mice and C57BL/6 mice were purchased from Taconic Farms and Jackson Laboratories, respectively. The NOD.Igμ^{-/-} mice were generously provided by Dr. David Serreze (Jackson Laboratories). All mice were housed in microisolators with filter tops on a ventilated rack under specific pathogen free conditions. Serological tests included those for mouse hepatitis virus, ectromelia virus, parvovirus, pneumonia virus, Sendai virus, lymphocytic choriomeningitis virus, Theiler's encephalomyelitis virus, enteric reovirus, minute virus of mice, epizootic diarrhea of infant mice, Mycoplasma pulmonis, pinworms, Aspiculuris spp, Syphacia spp and fur mites. Bedding was changed in laminar flow workstations and personnel wore protective disposable garments. Animals were fed standard lab chow and water *ad libitum*. Principles of laboratory animal care (NIH publication no. 85–23, revised 1985; <http://grants1.nih.gov/grants/olaw/references/phspol.htm>) were followed. The University of California, Los Angeles Animal Research committee approved all animal care and procedures.

Fostering

Newborn NOD mice were switched within 48 hours of birth onto a foster mother that had given birth within 48 hours. About 30 newborn NOD mice (of both sexes) were raised on about 7 dams of each mouse strain. The mice were weaned at 4 weeks of age.

Diabetes Diagnosis

Following weaning, only female mice were monitored for T1D. Urine glucose levels were checked weekly. After observing abnormal glucose in the urine, blood glucose levels were monitored twice weekly. Two consecutive blood glucose levels 300 mg/dL was considered as T1D onset. The mice were monitored until they were 45–52 weeks in age.

Statistics

Time to diabetes/percent with diabetes over time was computed using Kaplan-Meier survival (life table) methods. The p values for comparisons between groups were computed using the log rank test.

Results

Studies of postnatal exposure to islet autoantibodies through the milk

To examine whether postnatal transmission of islet autoantibodies through the milk plays a role in T1D pathogenesis, newborn NOD mice were switched within 48 hours after birth to other NOD or NOD.Igμ^{-/-} dams that had given birth within 48 hours. The adopted NOD mice are designated as NOD/NOD or NOD/NOD.Igμ^{-/-} mice, respectively. NOD.Igμ^{-/-} mice are B-cell deficient and do not develop T1D since B cells are critical APC for disease pathogenesis [14–17]. The NOD/NOD mice displayed a typical incidence of T1D, indicating that the cross-fostering procedure itself had no effect on their risk for developing T1D (Fig. 1). We observed that the elimination of antibodies from the mother's milk did not influence long-term diabetes incidence in NOD/NOD.Igμ^{-/-} mice (Fig. 1). Thus, autoantibodies in NOD mother's milk are not required for T1D development. This result contrasts with the results of Greeley et al., who found that NOD mice reared after embryonic implantation into NOD.Igμ^{-/-} mice had about one-half the disease incidence of those implanted into NOD mothers. This suggests that prenatal factors, but not postnatal factors, either present-, or absent from-, the NOD.Igμ^{-/-} maternal environment, protect NOD progeny from developing T1D. This protection may be due to eliminating prenatal exposure to islet autoantibodies, or other protective factors in the NOD.Igμ^{-/-} mothers resulting from their derivation by backcrossing from C57BL/6 mice (see Discussion).

We also cross-fostered newborn NOD.Igμ^{-/-} mice on NOD dams to determine whether postnatal transmission of islet autoantibodies was sufficient to cause disease in B cell-deficient NOD mice. None of the NOD.Igμ^{-/-}/NOD mice developed T1D, indicating that transmission of islet autoantibodies through the milk to B cell-deficient NOD mice is insufficient to promote T1D (Fig. 1).

The incidence of T1D in adopted NOD mice depends on the strain of the foster mother

Since the postnatal transmission of autoantibodies does not appear to contribute to T1D development, we next examined whether other postnatal maternal factors influence T1D incidence. It was previously shown that NOD mice arising from embryo implantation into ICR mice had a very reduced T1D incidence [13]. To further test how postnatal maternal factors affect T1D incidence in NOD mice, we reared newborn NOD mice on ICR, C57BL/6 or other NOD mouse dams. The adopted NOD mice were designated NOD/ICR, NOD/C57BL/6 or NOD/NOD mice, respectively. NOD/NOD mice had a typical incidence of T1D, again demonstrating that the cross-fostering procedure itself had no effect on their risk for developing T1D (Fig. 2). In contrast, NOD/ICR mice had a significantly reduced T1D incidence, with 25% developing T1D by 35 weeks in age, compared to 90% of those reared by foster NOD mothers (NOD/NOD mice) (Fig. 2). At 52 weeks in age, 56% of NOD/ICR mice had developed T1D compared to 90% of the NOD/NOD mice (p=0.001). Thus, postnatal maternal factors can have

a significant impact on long-term T1D incidence. The data shown in Figs. 1 and 2 came from mice that were raised about 1 year apart—yet the diabetes incidence in the control NOD/NOD group was similar in both studies, attesting to the uniformity of diabetes incidence in our colony over the course of the two year study.

Kagohashi et al., implanted NOD embryos into pseudopregnant ICR mice [13]. Only about 7% of their NOD/ICR mice developed T1D by 35 weeks in age, while 60% of their control NOD/NOD mice developed T1D. Thus, while we observed a significant reduction in T1D incidence from postnatal fostering newborn NOD mice on ICR mothers, it was not as great as the dramatic reduction reported after implanting NOD mouse embryos into ICR mothers. Apparently, both prenatal and postnatal maternal factors can modulate progeny's long-term risk for developing T1D.

In contrast to NOD/ICR mice, NOD/C57BL/6 mice developed T1D at the usual rate, similar to that of control NOD/NOD mice (Fig. 2). Thus, rearing NOD mice on non-autoimmune mothers differentially affected their T1D incidence, depending on the strain of the foster mother.

Discussion

Previous studies using embryo transfer have observed reduced T1D incidence in NOD mice reared from NOD.Igμ^{-/-}, NOD α-HEL, ICR and DBA mothers, suggesting that *in utero* transmission of islet autoantibodies plays an important role in subsequent T1D development [12,13,18]. These studies however, did not distinguish whether the elimination of autoantibody transfer *in utero*, postnatally (through milk), or other maternal factors, were important for preventing T1D. We examined whether the postnatal transmission of islet autoantibodies through milk affects T1D development.

We observed that NOD/NOD.Igμ^{-/-} mice had a normal T1D incidence, indicating that postnatally transmitted autoantibodies are not required for disease pathogenesis. Our finding that NOD/NOD.Igμ^{-/-} developed T1D at the usual rate, while a previous report found that NOD/NOD.Igμ^{-/-} and NOD/α-HEL NOD mice arising from embryo transfer had about one-half of the normal T1D incidence [12], suggests that the prenatal transmission of islet autoantibodies could be an important factor in T1D pathogenesis. However, other factors may contribute to the previously reported protection. The NOD.Igμ^{-/-} and α-HEL NOD transgenic mice were derived by crossing C57BL/6 mice with the Igμ^{-/-} mutation, or the HEL transgene, with NOD mice for 7–10 generations [12]. While the NOD.Igμ^{-/-} and α-HEL NOD mouse genomes should contain only a small percent of C57BL/6 genome, the mouse genome has hundreds of copies of different endogenous viruses, which are likely to differ in expression between NOD and C57BL/6 mice. In addition, even the small percent of C57BL/6 genome remaining after backcrossing encodes many genes, some of which may be differently expressed relative to NOD mice, and affect the maternal environment and fetus physiology in subtle ways. Finally, it has been shown that treating newborn NOD mice with mouse monoclonal antibodies (100 μg, ip) of the IgM subtype can efficiently prevent T1D [19]. Notably, the HEL antibody expressed by α-HEL NOD mice is IgM subtype and its transfer to NOD progeny may have contributed to the observed protection [12]. Accordingly, it is an open question whether the elimination of islet autoantibodies *in utero*, or other factors, reduced T1D incidence in the NOD mice arising after embryo transfer to NOD.Igμ^{-/-} and α-HEL NOD transgenic mice.

In addition, we observed that raising NOD.Igμ^{-/-} on NOD dams did not lead to any of the offspring developing T1D. Hence, transmission of islet autoantibodies to neonates that are B cell-deficient, is insufficient for T1D development. This is not unexpected as the APC function

of B cells is crucial for the spreading of T cell autoimmunity and progression to T1D [14–17].

As postnatal islet autoantibody transmission does not appear to play a role in T1D development, we next tested whether there were other protective factors associated with raising NOD mice postnatally on nonautoimmune mothers. We used ICR dams as foster mothers since a previous study showed that the NOD mice arising from embryo transfer into ICR mice have a very low incidence of T1D [13]. We also reared NOD mice on C57BL/6 dams as second nonautoimmune strain.

We observed that fostering newborn NOD mice on ICR mothers resulted in a reduced long-term diabetes incidence. The T1D incidence in NOD/ICR mice at 40 weeks in age was significantly less than that in control NOD/NOD mice (50% vs. 90%). This protection for T1D is not as great as that reported after NOD mice were transferred as embryos to ICR mice (7% in NOD/ICR vs. 60% in NOD/NOD mice at 40 weeks in age, the latest time point in that study, [13]). These results suggest that both the prenatal and postnatal ICR maternal environment provides protective factors and/or is deficient in factors that increase disease risk. In contrast to the reduced T1D incidence in NOD/ICR mice, NOD/C57BL/6 mice developed T1D at essentially the same rate as NOD/NOD mice.

What could be the basis for the reduced T1D incidence in NOD/ICR vs. NOD/C57BL/6 mice? Kagohashi et al. reported that NOD mice arising from embryo transfer to ICR mothers developed insulinitis earlier than their NOD/NOD counterparts [13]. Apparently, immunostimulatory factors in the ICR mother promoted a precocious, but benign, insulinitis before 3 weeks in age. This observation has parallels with our findings in NOD mice that were protected from T1D by neonatal administration of an antigen-based immunotherapy. We found that neonatal immunization of NOD mice with any β -cell antigen (ip, in incomplete Freund's adjuvant) lead to the precocious appearance of T cell autoimmunity to all of the tested β -cell autoantigens by 4 weeks in age, far in advance of when autoimmunity would have naturally arisen to most of the antigens [20]. Evidently, young NOD mice have a broad array of β -cell reactive T cells whose activation/expansion can occur rapidly in response to the immunostimulatory environment created by treatment with a single β -cell autoantigen. Similarly treated non-autoimmune mouse strains developed immune responses only to the injected antigen. Thus, neonatal immunostimulation can induce the precocious appearance of a broad array of benign autoreactive T cell responses in mice genetically susceptible to developing β -cell autoimmunity. We suspect that the precocious and more extensive insulinitis observed in NOD progeny arising from embryo implantation in nonautoimmune mothers is evidence of an early immunostimulatory environment, similar to the precocious benign T cell autoimmunity that we observed after neonatal antigen-based therapy. These early immune responses can greatly influence the phenotype of much later immune responses [21–23].

Kagohashi et al. also found that there was an earlier expression of xenotropic retroviruses and higher levels of corticosterone levels in NOD/ICR mice than in NOD/NOD mice [24]. They suggested that endogenous virus and maternal factors such as corticosterone and leptin may account for the reduced T1D incidence in NOD/ICR mice [13,24]. Others have pointed to the transfer of insulin, other hormones and growth factors through the placenta or the milk, as candidate modulators of T1D incidence in humans [4,25,26]. Milk also contains antimicrobial agents, prostaglandins, interleukins, TNF α and TGF β [27]. Casein is another milk protein that has been suspected to induce islet-reactive T cell responses [28]. However, we have found that oral administration of a non-self casine fraction fails to induce casine-reactive T cell responses in NOD mice (unpublished observation). Differences in the gut flora of the dams, as well as in the nutrient composition of their milk, could have also impacted the diabetes incidence of the foster mice. In addition, the extent of maternal care can have a lasting effect on offspring's

subsequent immune responses [29–31]. Thus, there are numerous maternal factors that may contribute to the protection observed in our NOD/ICR, but not NOD/C57BL/6 mice—too many unravel without extensive efforts. Presumably, such factors could also have influenced previous studies of NOD mice arising from embryo transfer [12,13,18].

There are several lines of evidence that in humans, maternal transmission of autoantibodies does not contribute to offspring's risk, and may even be protective. The children of diabetic females have a lower risk for T1D than the children of diabetic males--the opposite of what would be expected if maternal transmission of islet antibodies contributes to T1D development [32]. Moreover, transient high β -cell autoantibody levels in human newborns is not associated with increased risk for T1D [5,6]. Finally, fetal exposure to islet autoantibodies in children born to mothers with T1D may be protective [7]. Therefore, if transmission of islet autoantibodies *in utero* is a risk for T1D in NOD mice, this finding may not extend to the human situation.

The increasing incidence of T1D worldwide over the last decades underscores the important role that environmental factors can play on T1D incidence. The notion that T1D incidence might be reduced through modulating exposures to environmental and maternal factors is an enticing strategy for promoting community health. Our findings, as well as that of others, suggest that commonplace maternal factors other than islet autoantibodies can modulate T1D incidence in offspring. A better understanding of these early influences on T1D development may help identify the causes of the increasing incidence of T1D worldwide, and aide in the design of possible interventions.

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Abbreviations

HEL	hen egg lysozyme
ICR	Institute of Cancer Research
NOD	nonobese diabetic mice
T1D	type 1 diabetes

Figure 1.

Elimination of autoantibodies in NOD milk has no effect on subsequent disease incidence, and neonatal acquisition of islet-autoantibodies through milk is insufficient to elicit T1D in B cell-deficient mice. Newborn NOD mice were fostered on NOD.Igμ^{-/-} dams or other NOD dams. In addition, newborn NOD.Igμ^{-/-} mice were reared on NOD dams. After weaning, female NOD mice were monitored for T1D development for up to 45 weeks in age. Data shown is the diabetes incidence for NOD/NOD (◆), NOD/NOD.Igμ^{-/-} (□), and NOD.Igμ^{-/-}/NOD mice (Δ) (N= 15, 13 and 7 mice/group, respectively).

Figure 2.

NOD pups fostered on ICR, but not C57BL/6, mothers have reduced disease incidence. NOD mice were switched within 48 hours of birth onto an ICR (NOD/ICR), C57BL/6 (NOD/C57BL/6), or another NOD (NOD/NOD), dam that had given birth within 48 hours. At 4 weeks in age, mice were weaned, and female NOD mice were monitored for T1D development for up to 45–52 weeks in age. Data shown is the diabetes incidence for NOD/ICR (□), NOD/C57BL/6 mice (Δ), and NOD/NOD (◆) (N= 16, 10 and 10 mice/group, respectively)