Pregnancy Hormones Prevent Diabetes and Reduce Lymphocytic Infiltration of Islets in the NOD Mouse

Illani Atwater,1,2 Bernard Gondos,1 Ray DiBartolomeo,1 Rodrigo Bazaes,1,3 and Lois Jovanovic1
1Sansum Medical Research Institute, Santa Barbara, California, USA; 2Instituto de Nutricion y Tecnologia de Alimentos, Santiago, Chile
3Faculty of Medicine, University of Chile, Santiago, Chile

Abstract. Pregnancy is associated with a depression of the immune inflammatory system, and with increased growth and function of the pancreatic islets of Langerhans. We monitored glucosuria, blood glucose concentration, and lymphocytic infiltration of pancreatic islets in 30 female, 10-wk-old, pre-diabetic non-obese diabetic (NOD) mice divided into 3 treatment groups for 13 wk: group 1, saline; group 2, pregnancy hormones (dexamethasone 4 mg/Kg/day, progesterone 1.7 mg/Kg/day, growth hormone 0.6 mg/Kg/day, prolactin 1 mg/Kg/day, and estradiol 0.05 mg/Kg); and group 3, prolactin alone (1 mg/Kg/day). At sacrifice, the pancreases were fixed in paraformaldehyde and islet infiltration was evaluated. In the saline-treated group (#1) 4/10 mice developed diabetes, while in the hormone treated group (#2) none of the mice developed diabetes. Only 1/10 mice in the prolactin-treated group (#3) developed diabetes during the study. Islets from the hormone cocktail treated group were significantly less infiltrated than islets from the other 2 treatment groups (p <0.001). Thus, the pregnancy hormones protected NOD mice from developing diabetes and significantly reduced or eliminated insulitis and islet infiltration. Prolactin alone had a partial protective effect. The results have implications for prevention of type 1 diabetes and for immune suppression in patients receiving islet cell transplantation. (received 20 September 2001, accepted 2 November 2001)

Keywords: prolactin, progesterone, growth hormone, placental lactogen, estradiol, dexamethasone, glucose, immune suppression, islet cell transplantation

Introduction

During pregnancy, the survival of a genetically disparate allogeneic mammalian conceptus contradicts the laws of tissue transplantation. The immunological paradox of fetal growth despite the genetic disparity remains an enigma. Solving the paradox may lead to mechanisms that contribute to the field of islet transplantation.

Pregnancy is associated with a depression of the immune inflammatory system, and several autoimmune diseases have been observed to go into remission during pregnancy [1,2]. The placenta produces immune suppressing hormones to prevent a maternal lymphocytic response to the fetal/placental unit [3-5]. In addition, hormones elevated during pregnancy have been reported to increase islet growth and function [6-14]. In vitro studies have ascribed most of the islet growth effects during pregnancy to the hormone prolactin [15-19]. There are recent clinical data to suggest that women with type 1 diabetes begin to make their own insulin during pregnancy [20-22], which suggests that not only the immune mediated destruction of beta cells is depressed during pregnancy, but also that beta cells are regenerated by the hormonal changes. Thus, pregnancy hormones may hold promise as part of the cure for type 1 diabetes.

To test this hypothesis, we treated non-obese diabetic (NOD) mice with pregnancy hormones. The NOD mouse is an animal model for type 1, or autoimmune, diabetes, since the females spontaneously develop an autoimmune form of diabetes.
between about age 12 wk and 6 mo [23,24]. In this study, we report that injecting pregnancy hormones in pre-diabetic female NOD mice prevents diabetes and substantially diminishes lymphocytic infiltration of the islets of Langerhans.

Materials and Methods

Female NOD mice, ages 7 to 10 wk, were divided into 3 groups of 10 mice each, with 1 mouse per cage and mice from the same litter divided among the groups. Group 1 received daily ip injections for 13 wk of saline; group 2 received daily ip injections for 13 wk of saline containing a mixture (“cocktail”) of pregnancy hormones (dexamethasone, 4 mg/Kg/day; progesterone, 1.7 mg/Kg/day; growth hormone (to mimic placental lactogen), 0.6 mg/Kg/day; prolactin, 1 mg/Kg/day; and estradiol, 0.05 mg/Kg); and group 3 received daily ip injections of saline with prolactin alone (1 mg/Kg/day) for 8 wk and then no treatment for 5 wk. The hormones were added to sterile saline solution at concentrations such that all mice received the same volume of injection fluid. All hormones were purchased from Sigma Co. (St. Louis, MO) in water-soluble form.

Mice were weighed weekly. Each mouse was placed in a metabolic cage for several hr on 3...
mornings per wk (Monday, Wednesday, Friday) and the collected urine was tested for glucose. Animals that tested positive for glucosuria had blood glucose concentration determined. This was done by snipping off the tip of the tail (two min after topical lidocaine application), collecting a drop of blood directly onto a glucose reagent strip, and analyzing the blood immediately with a reflectance meter (One-Touch Glucose Meter, LifeScan, Inc., Milpitas, CA). Pre-diabetic female NOD mice had non-fasted blood glucose of 94.2±20.4 mg/dl (mean±SD, n = 25), measured in the afternoon.

Mice showing blood glucose concentrations >2 SD above the mean (ie, >134 mg/dl) were diagnosed as diabetic. Mice showing blood glucose concentrations of >400 mg/dl during the study were sacrificed. The two-sided Fisher's Exact Test [25] was used to evaluate the significance of differences in diabetes onset among the study groups. After the 13-wk experimental period, all mice were killed by injection of ketamine.

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Sansum Medical Research Institute. The study conforms to NIH guidelines for the use of animals for biomedical research.

Upon sacrifice, pancreatic tissues were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Specimens were examined by one of the authors (B.G.) in a blinded manner. All specimens were evaluated for the degree of islet infiltration by lymphocytes. A semi-quantitative scheme was used as follows: 0, no infiltration; +, periinsulitis; ++, moderate infiltration; ++++, extensive infiltration and islet destruction or diabetes. Illustrations of the different degrees of involvement are shown in Fig. 1. Analysis of the islet infiltration was done using the Chi-square test for linear trend (SAS® for Windows, Version 8, SAS Institute, Inc., Cary, NC).

**Results**

The mice weighed 26.1±2.0 g at the start of the study and 27.1±2.2 g at the end of the study; there were no significant changes of weight in any of the 3 groups during the study.

After 3 wk of treatment, some mice transiently developed glucosuria (saline treated, 5/10; hormone cocktail treated, 1/10; prolactin treated; 2/10), which reversed within 5 days. None of the mice had blood glucose values >134 mg/dl at that time. After 6 wk of treatment, 2 mice in the saline group again showed glucosuria and 1 developed hyperglycemia, with blood glucose >400 mg/dl. This mouse was sacrificed. After 10 wk of treatment, 2 additional mice in the saline group showed positive glucosuria and developed blood glucose >400 mg/dl and were also sacrificed.

Blood glucose measured in non-diabetic NOD mice at the end of the study (nonfasting, in the afternoon) averaged 97±7.3 mg/dl (n=6) in the saline group, 91.5±10.5 mg/dl (n=10) in the hormone treated group, and 100.4±22.2 mg/dl (n=10) in the prolactin treated group. The mean blood glucose concentrations were not significantly different among the different study groups. One of the saline treated mice had blood glucose concentration of 147 mg/dl and 1 of the prolactin treated mice had blood glucose of 236 mg/dl at the end of the study, and they were thus considered to be diabetic. None of the mice in the hormone cocktail treated group developed blood glucose concentrations >134 mg/dl during the study.

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<th>Treatment</th>
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a 0, no infiltration; +, periinsulitis only; ++, moderate infiltration into islets; ++++, extensive infiltration with islet destruction or diabetes.
b See Methods section for composition of hormone cocktail.
In summary, 4/10 mice in the saline treated group, 1/10 mice in the prolactin treated group, and none of the pregnancy hormone treated group developed diabetes.

Table 1 shows the degree of islet infiltration by lymphocytes in the different study groups. Only 6 of 10 pancreases from the saline group could be evaluated by the pathologist since 3/10 animals in this group were sacrificed because of severe diabetes (blood glucose >400 mg/dl) before the end of the study and 1 was hyperglycemic (blood glucose = 147 mg/dl) at the end of the study and did not have sufficient islets for appraisal (all included as +++ in Table 1). The mouse showing the highest degree of infiltration in the prolactin treated group was the mouse diagnosed with diabetes (blood glucose 236 mg/dl). It can be seen that, while the saline and prolactin treated groups showed similar levels of infiltration, the islets from mice treated with the mixture of pregnancy hormones showed less infiltration. Analysis using the Chi-square test for linear trend showed a significant reduction in infiltration in the hormone treated group compared to the saline group (p < 0.001). A similar analysis, not taking into consideration the 4 diabetic animals in the saline group, also showed a significant difference between the saline treatment and hormone cocktail treatment (p <0.01). All animals in the saline group showed some degree of islet involvement. Three of 10 mice in the group treated with pregnancy hormones showed no islet infiltration and no periinsulitis. Only 1 animal had infiltration into islets.

Discussion

The studies presented here show that injection of pregnancy hormones prevents diabetes and significantly reduces islet infiltration in the NOD mouse. Prolactin alone also protected against diabetes, but did not significantly reduce lymphocytic infiltration into the islets. Two hypotheses emerge from this observation. First, enhanced islet growth can balance islet destruction, and, second, prolactin protects beta cells from cell death, either through effects on the immune system or through effects on the beta cells themselves.

Prolactin has been shown in vitro to enhance cell-to-cell coupling within the islet [16] and has also been implicated in the increased islet growth, increased insulin content of beta cells, and increased glucose sensitivity observed in maternal islets during pregnancy [14-17]. Thus, it is possible that the action of prolactin on beta cells may also protect them from immune destruction. This theory would explain why we observed infiltration of islets in the animals treated with prolactin similar to that seen in the sham treated animals without comparable onset of diabetes.

It is interesting that many animals in the group treated with pregnancy hormones had periinsulitis without lymphocytic invasion into the islets. The limited peripheral lymphocytic response suggests the possibility of an immunologic reaction that was prevented from further progression. If, indeed, the pregnancy hormones were responsible for limiting the degree of lymphocytic infiltration, as the findings appear to indicate, that would imply a role in preventing islet destruction.

Islet infiltration is thought to progress from peri-insulitis to partial infiltration of the islet and finally to total infiltration and islet destruction. It is uncertain that peri-insulitis or partial islet infiltration affects the insulin secreting activity of the islet. As islets are destroyed, however, it may be expected that the animals will first become glucose intolerant, and then hyperglycemic and diabetic. The paradoxical increase in urine glucose observed in some animals, when no increase in blood glucose was detected, possibly reflects an overnight accumulation of glucose. Since mice usually feed at night, postprandial blood glucose could have been elevated, leading to delayed glucosuria, but the blood glucose could have returned to normal by the time it was measured (between 11 am and 1 pm). Detection of glucosuria may have been a better indicator of transient postprandial hyperglycemia than a momentary blood glucose determination. Thus, the transient occurrence of glucosuria may indicate that islets were partially destroyed, and then spontaneously recovered. The recovery was enhanced in the hormone treated groups, in agreement with the observation that islet growth is enhanced during pregnancy.
Relatively few animals developed diabetes in the course of this study, i.e., 4 of 10 by age 5 mo. This rate is equal to the background rate of diabetes in the Sansum NOD colony. Although not statistically significant because of the small number of mice used in this study, the results suggest that there was a difference in diabetes onset among the different groups, the control group (4/10), the prolactin treated group (1/10), and the hormone-injected group (0/10) (p = 0.087 for saline versus hormone cocktail treated group).

The infiltration score of the prolactin-treated group, 5 wk after cessation of treatment, was not significantly different from the saline-treated group, and only 1 mouse in the prolactin treated group showed elevated blood glucose. Since prolactin has been shown to induce islet growth [17], our results suggest that perhaps in the prolactin treated group, islets were growing fast enough to “keep ahead” of the islet destruction process.

We observed in this study a significant reduction of infiltration in islets of mice treated with pregnancy hormones. Significantly, 3/10 of these mice showed no infiltration, while in the saline and prolactin treated groups, all mice showed some degree of infiltration.

In summary, we have established that pregnancy hormones prevent the onset of autoimmune diabetes and greatly reduce islet infiltration in the NOD mouse. The results have important implications for the development of a treatment for diabetes in patients, for the development of new immune suppression regimes after islet transplantation, and for the understanding of the effects of pregnancy on autoimmune diseases in general.

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References


