Glucocorticoid Receptor Expression Is Altered in Pancreatic β Cells of the Non-obese Diabetic Mouse During Postnatal Development

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Glucocorticoids play a crucial role in the regulation of carbohydrate metabolism and in the immune response, and can influence the development of diabetes in certain animal models including autoimmune type 1 diabetes in the non-obese diabetic (NOD) mouse. In these animals, the onset of destructive autoimmune pancreatic changes (insulitis) occurs at around 3 weeks of age. Moreover, the incidence of diabetes is significantly higher in females compared to males. However, the underlying mechanisms for this sex-specificity are unknown. Therefore, the present study was undertaken to examine the expression of the glucocorticoid receptor (GR) in pancreatic islets of Langerhans of the NOD mouse during the first 3 weeks of postnatal development. Immunohistochemistry was used to determine pancreatic GR expression and to identify insulin-secreting β cells in postnatal (1-, 2-, and 3-week-old) NOD mice. Age-matched NOD.SCID mice (immunodeficient animals with the same NOD genetic background) were used as control animals. In both strains, regardless of sex or age, GR staining was found predominantly in the cytoplasm of β cells but was also present in other cell types within the islets. At all ages, the percentage of islet cells containing GR was similar between male and female animals of the same strain. In control mice, the percentage of islet cells containing GR increased progressively from 80% at 1 week of age to 100% at 3 weeks of age. In marked contrast, in the NOD mice, the proportion of islets containing GR decreased from 95% at week 1 to only 60% at 3 weeks of age. We conclude that sex-specific differences in the incidence of diabetes are not associated with altered pancreatic GR expression in NOD mice during early postnatal development. However, the distinct and remarkable decrease in islet GR levels at 3 weeks of age may contribute to the onset of insulitis, and potentially to the ontology of diabetes in NOD mice, as a result of the loss of protective immunosuppressive effects of glucocorticoids.

GLUCOCORTICOIDS play a crucial role in the regulation of carbohydrate metabolism: they increase hepatic glucose production through gluconeogenesis by induction of enzymes such as glucose-6-phosphate and phosphoenolpyruvate.[1-3] In this regard, glucocorticoids are known to be hyperglycemic agents aggravating diabetes. Conversely, these steroid hormones are also anti-inflammatory and immunosuppressive agents, capable of limiting the progression of autoimmune diseases such as insulin-dependent diabetes mellitus (IDDM) or type 1 diabetes (T1D).[4-6] Furthermore, glucocorticoids have been shown to influence the onset of diabetes in the non-obese diabetic (NOD) mouse, an animal which spontaneously develops T1D early in life in a manner similar to humans. In these animals, at around 3 weeks of age the prestage of diabetes, known as insulitis, is characterized by infiltration of the pancreatic β cells by immune cells.[7] It has previously been demonstrated that NOD mice subjected to stress had a delayed onset of diabetes compared to control animals. Conversely, the onset and incidence of diabetes were more pronounced in adrenalectomized NOD mice compared to their adrenal-intact counterparts.[8] Moreover, the incidence of diabetes is much higher in female NOD mice compared to their male littermates[9]; at 25 weeks of age, approximately 90% of females have developed diabetes compared to only about 30% of males. The underlying mechanisms for this sex-specificity are unknown.

Glucocorticoid actions are mediated predominantly through their specific cytoplasmic receptors which, upon binding the ligand, translocate to the nucleus where they interact with specific DNA elements and/or other transcription factors to induce or repress the expression of target genes.[10,11] The glucocorticoid receptor (GR) is ubiquitously expressed in rodents,[12] including pancreatic islets of the mouse,[13] where its level of expression is an important determinant of glucocorticoid activity.

Given the role of glucocorticoids in the development of diabetes in the NOD mouse, we hypothesized that altered GR expression may occur in the pancreatic islets of the NOD mouse at critical stages of early postnatal development. We further hypothesized that the sex-specific differences in the incidence of diabetes in NOD mice may be associated with sex-specific alterations in islet GR levels during critical stages of postnatal development. The present study was therefore undertaken to examine these hypotheses.

MATERIALS AND METHODS

Tissue Collection

Virgin NOD females 4 to 5 weeks of age and male mice were obtained from the breeding colony at the J.P. Robarts Research Institute (London, Ontario, Canada) and were allowed free access to food and water. Animals were housed in the Animal Care Facility at the Lawson Health Research Institute, and maintained at 25°C on a 12-hour light/12-hour dark cycle in a pathogen-free environment. At 8 weeks of age, breeding pairs were caged in individual cages and females were checked daily for vaginal plugs. The males were left with the females over the whole pregnancy. Female and male pups were sexed and then killed at 1, 2, and 3 weeks of age. At 7 days, mice were decapitated and at 2 to 3 weeks of age were axfixiated with CO_2. Age-matched

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NOD.SCID mice (immunodeficient animals that do not have functional T or B lymphocytes, with the same NOD genetic background), used as the control animals, were also obtained from the breeding colony at J.P. Robarts Research Institute. All procedures were approved by the Animal Care Committee of the University of Western Ontario in accordance with the guidelines of the Canadian Council on Animal Care. Pancreata were removed and fixed by immersion with 4% paraformaldehyde and 0.02% gluteraldehyde in 70 mmol/L phosphate buffer, pH 7.0, at 4°C for 24 hours, washed twice daily with phosphate buffer for 48 hours, which was then changed into 70% ethanol. They were then embedded in paraffin, and 5-μm sections were prepared by standard methods and mounted onto microscope slides (Superfrost Plus, Fisher Scientific, Unionville, Ontario, Canada).

Immunohistochemistry

After deparaffinization and rehydration, serial tissues sections were incubated sequentially in 1% hydrogen peroxide for 10 minutes to quench endogenous peroxidase activity and then in 10% normal swine serum for 30 minutes. Tissue sections were incubated at 4°C overnight. in rabbit polyclonal anti-GR antiserum capable of binding to both cytoplasmic and nuclear GR (1:300; Santa Cruz Biotech, Santa Cruz, CA) and rabbit antimouse insulin antiserum (1:30; to identify β cells), glucagon antiserum (1:100; to identify α cells), and somatostatin antiserum (1:1,000; to identify δ cells). Insulin, glucagon, and somatostatin antiserum were kindly donated by Dr Tom McDonald (University of Western Ontario). As a control for each antibody, sections were incubated with 10-fold excess concentrations of absorbed antiserum at 4°C overnight. Sections were immunostained using an avidin-biotin-peroxidase method (LSAB Plus kit; Dako, Santa Barbara, CA), with 3,3-diaminobenzidene as the chromagen. Slides were counterstained with methyl green (Dako) and mounted with permount (Fisher Scientific).

Morphometric Analysis

Morphometric analysis was performed using a Carl Zeiss transmitted microscope at a magnification of 250x or 400x. Analysis of the pancreatic sections was performed with a Northern Eclipse version 2.0 morphometric analysis software (Empix Imaging, Mississauga, Ontario, Canada). Immunostained cells were analyzed by image analysis and selected by red, blue and green (RBG) threshold.

Statistical Analysis

For each individual islet, the percentage islet expressing GR was calculated in each group. Data are presented as the mean ± SEM. Statistical comparisons were made using Student's unpaired *t* test to assess differences between control and NOD animals of the same sex and age, and between female and male mice of the same age and strain. Statistical significance was taken as $P < .05$. In all groups, n = 8 to 10 animals with an average of 100 islets per group.

RESULTS

Tissue sections were prepared from pancreata collected from NOD and control mice at 1, 2, and 3 weeks of age, and analyzed for GR protein expression by immunohistochemistry. In both strains, regardless of sex or age, GR staining was found predominantly in the cytoplasm of β cells (Figs 1 and 2,) but was also present in other cell types within the islets (data not shown). At all ages, the percentage of islet cells containing GR was similar between male and females of the same strain (Fig 3). In control mice, islet GR expression progressively increased from 80% at 1 week of age to approximately 100% at 3 weeks of age (Figs 1 and 3). In contrast, in the NOD mice, islet GR expression decreased from 95% at 1 week of age to only 60% at 3 weeks of age (Figs 2 and 3; $P < .01$ compared to 3-week-old control animals). The specific mmunohistochemical staining was abolished after absorption of antiserum with 10-fold excess concentration of GR, insulin, glucagon, and somatostatin (data not shown).

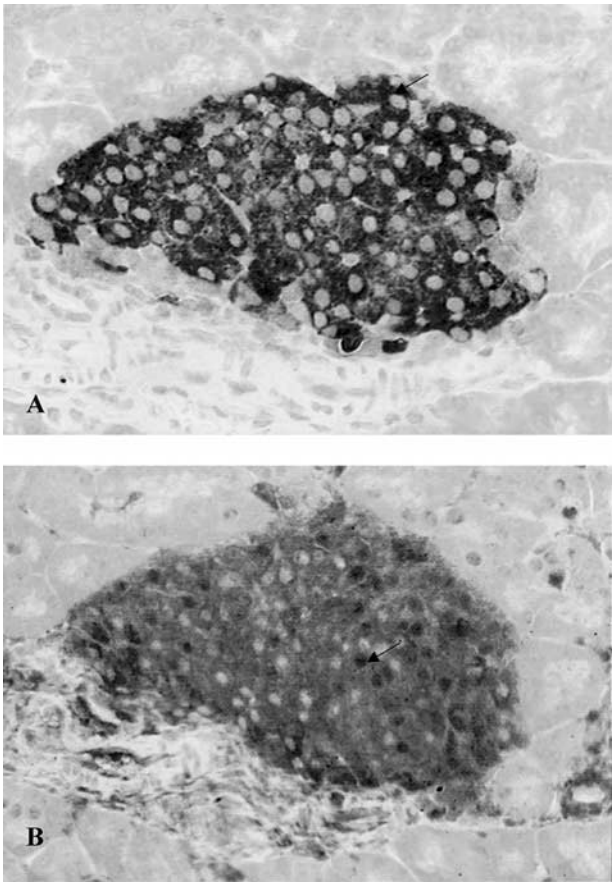

Fig 1. Representative microphotographs of (A) insulin (to identify β cells) and (B) GR localization in pancreata of 3-week-old female control (NOD.SCID) mice (original magnifications × 500). Arrows show cytoplasmic staining for insulin and some nuclear staining for GR. Immunohistochemistry using insulin and GR antiserum was performed on serial sections as described in the Methods.

DISCUSSION

In the present study, we have demonstrated for the first time that GR is expressed predominantly in β cells of the pancreas in the NOD mouse during postnatal development. Moreover, the level of GR expression in pancreatic β cells of the NOD mice decreases dramatically at 3 weeks of age when insulitis occurs, suggesting that the altered GR levels may contribute to the ontology of diabetes in these animals.

The findings of the current study show that in both strains, regardless of sex or age, GR staining was found predominantly in the cytoplasm of β cells but was also present in other cell types within the islets. This is consistent with other studies demonstrating the presence of GR in the endocrine > acinar > ductal cells of the adult rat pancreas.[14] Similarly, functional GR have been demonstrated in β cells of adult mice.[13] Taken

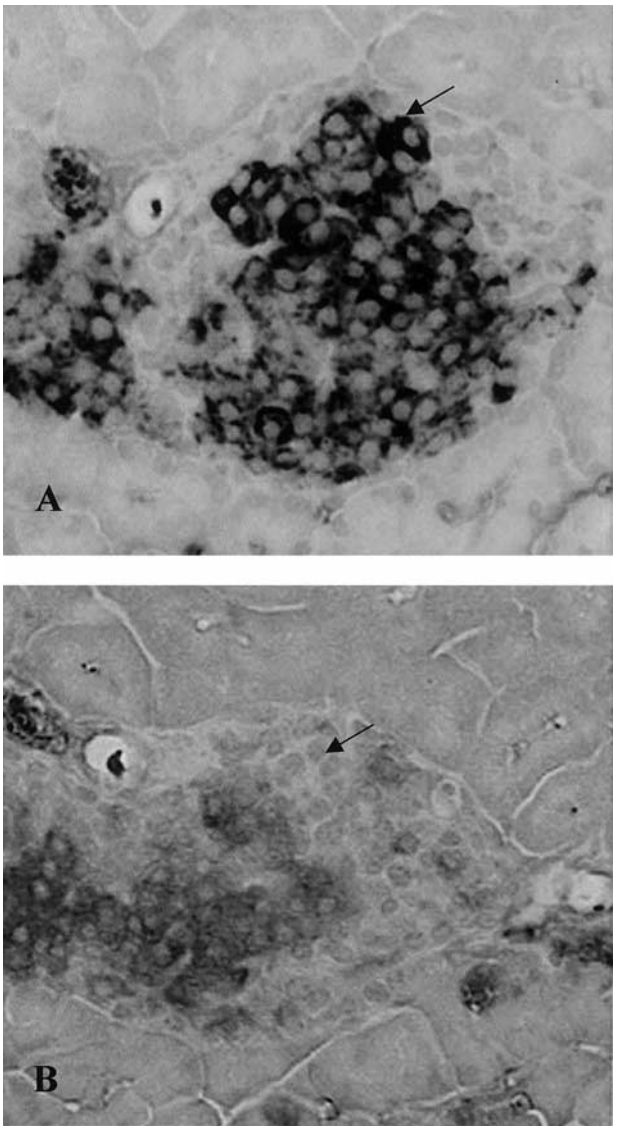

Fig 2. Representative microphotograph of (A) insulin (to identify β cells) and (B) GR localization in pancreata of 3-week-old female NOD mice (original magnifications × 400). Arrows show absence of GR staining compared to insulin staining in β cells. Immunohistochemistry using insulin and GR antiserum was performed on serial sections as described in the Methods.

together, these findings suggest that the β cell is the primary target of glucocorticoid action within the mouse pancreas.

The incidence of diabetes in the NOD mouse varies between animals, even between littermates housed together.[9] The disease is much more common in female mice compared to male mice. In our present colony, the onset of diabetes is earlier than usual: approximately 80% to 90% of female NOD mice have developed diabetes, compared to only 20% in males at 15 weeks of age. This sexual dimorphism is attributable, at least in part, to circulating sex hormones since neonatal gonadectomy increases the incidence of diabetes in males while reducing it in females.[15] However, the exact mechanisms by which these hormones elicit sex-specificity on the development of diabetes in NOD mice have yet to be elucidated. Given the ability of glucocorticoids to influence the development of diabetes in NOD mice,[8] we hypothesized that differences in islet GR expression between male and female NOD mice during the first 3 weeks of life may be a contributing factor to the observed sexual dimorphism. However, at all ages studied, the percentage of islet cells containing GR was similar between male and females of the same strain, suggesting that islet GR expression during early postnatal development is not associated with the sex-specific incidence of diabetes in NOD mice.

Nevertheless, strain-specific changes in pancreatic GR levels are evident in the postnatal mouse. In control animals, islet GR levels progressively increased from 80% at 1 week of age to 100% at 3 weeks of age while, by marked contrast, in the NOD mice, islet GR levels decreased from 95% at week 1 to only 60% at 3 weeks of age. Given that insulitis occurs at 3 weeks of age, the present findings suggest that the decreased pancreatic GR may contribute to the onset of insulitis. Furthermore,

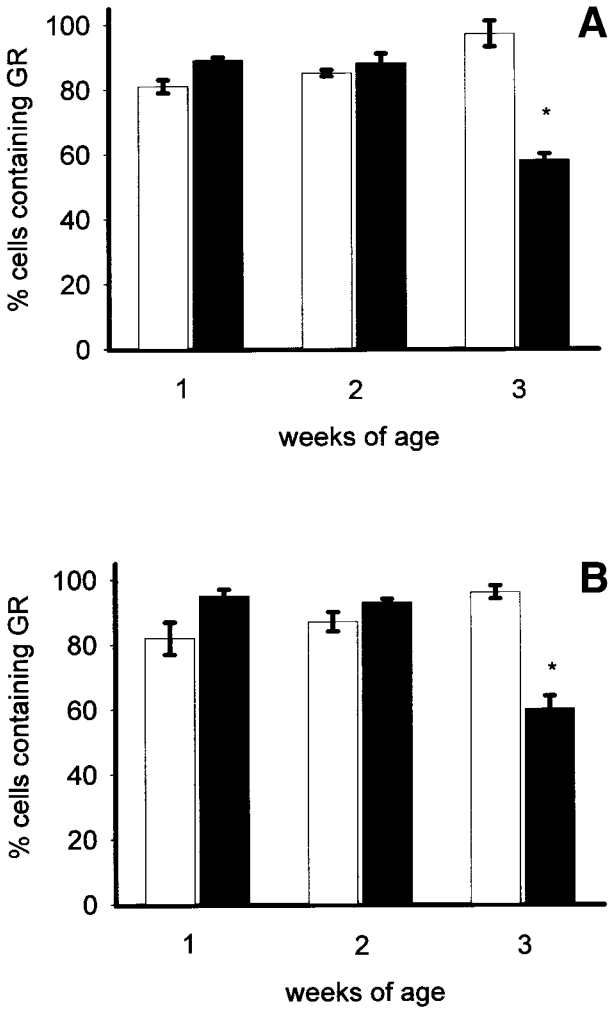

Fig 3. Percentage of pancreatic islets expressing GR in (A) female and (B) male control (NOD.SCID; □) and NOD (■) mice at 1, 2, and 3 weeks of age.

since decreased GR could result in the loss of protective anti-immune effects of glucocorticoids at this critical period,[16,17] it is conceivable that the decreased GR levels at 3 weeks of age may be a contributing factor in the development of diabetes in this animal. It is noteworthy that a study by Delaunay et al has shown that the pancreaticβ cell is an important target for the diabetogenic action (type 2 diabetes) of glucocorticoids in the mouse.[13]

At present, the mechanisms by which these changes in GR expression are brought about are unknown. An elevation in intrapancreatic immune cells seems the most obvious candidate. However, to the best of our knowledge it is currently unknown whether the immune cells involved in the autoimmune destruction, notably, the interleukins and T cells,[16,17] regulate GR expression. Likewise, it is unknown whether pancreatic concentrations of glucocorticoids, a well-known down-regulator of GR, are elevated in these 3-week-old NOD mice. Further studies are required to establish not only the mechanisms by which this decrease in GR localization is elicited, but also the functional significance of reduced pancreatic GR expression in the ontogeny of diabetes in NOD mice.

In summary, the experiments described here demonstrate that altered islet GR levels are not associated with the sex-specific development of diabetes in the NOD mouse. However, the dramatic decrease in islet GR levels at 3 weeks of age may contribute to the onset of insulitis, and potentially the development of diabetes, in NOD mice as a result of the loss of the protective immunosuppressive effects of glucocorticoids.

REFERENCES

1. Lenzen S, Bailey CJ: Thyroid hormones, gonadal and adrenal steroids and the function of the islets of langerhans. Endocr Rev 5: 411-434, 1984

2. McMahon MJ, Gerich J, Rizza R: Effects of glucocorticoids on carbohydrate metabolism. Diabetes Metab Rev 4:17-30, 1988

3. Surwit RS, Schneider MS, Feinglos MN: Stress and diabetes mellitus. Diabetes Care 15:1413-1422, 1992

4. Homo-Delarche F, Fitzpatrick F, Christeff N, et al: Sex steroids, glucocorticoids, stress and autoimmunity. J Steroid Biochem Molec Biol 4:619-637, 1991

5. Sternberg EM, Chrousos GP, Wilder RL, et al: The stress response and the regulation of inflammatory disease. Ann Intern Med 117:854-866, 1992

6. Wick G, Hu Y, Schwarz SG, Kroemer G: Immunoendocrine communication via the hypothalamo-pituitary-adrenal axis in autoimmune disease. Endocr Rev 14:539-563, 1993

7. Delovitch TL, Singh B: The non obese diabetic mouse as a model of autoimmune diabetes: Immune dysregulation gets the NOD. Immunity 7:727-738, 1997

8. Saravia-Fernandez F, Durant S, Hasnaoui AE, et al: Environmental and experimental procedures leading to variations in the incidence of diabetes in the non obese diabetic (NOD) mouse. Autoimmunity 24:113-121, 1996

9. Durant S, Coulaud J, Amrani A, et al: Effects of various environmental stress paradigms and adrenalectomy on the expression of autoimmune type 1 diabetes in the nonobese diabetic (NOD) mouse. J Autoimmun 6:735-751, 1993.

10. Evans RM: The steroid and thyroid receptor superfamily. Science 240:885-889, 1988

11. Beato M: Gene regulation by steroid hormones. Cell 56:335-344, 1989

12. Cole TJ, Blendy JA, Monaghan AP, et al: Molecular genetic analysis of glucocorticoid signalling during mouse development. Steroids 60:93-96, 1995

13. Delaunay F, Khan A, Cintra A, et al: Pancreatic ß-cells are important targets for the diabetogenic effects of glucocorticoids. J Clin Invest 100: 2094-2098, 1997

14. Matthes H, Kaiser A, Stier U, et al: Glucocorticoid rececptor gene expression in the exocrine and endocrine rat pancreas. Endocrinology 135:476-479, 1994

15. Hawkins T, Gala RR, Dunbar JC: The effect of neonatal sex hormone manipulation on the incidence of diabetes in nonobese diabetic mice. Proc Soc Exp Biol Med 202:201-208, 1993

16. Serreze DV, Leiter EH: Genetic and pathogenic basis of autoimmune diabetes in NOD mice. Curr Opin Immunol 6:900-906, 1994

17. Tochino T: The NOD mouse as a model of type 1 diabetes. CRC Crit Rev Immunol 8:49-81, 1987