Rapid Publications

Lymphocytic Thyroiditis and Diabetes in the BB/W Rat

A New Model of Autoimmune Endocrinopathy

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SUMMARY

The Bio Breeding/Worcester (BB/W) rat develops spontaneous insulin-dependent diabetes mellitus secondary to lymphocytic infiltration and destruction of the pancreatic beta-cells. This destructive process in the pancreas has been postulated to be based on a thymus-dependent cell-mediated autoimmune process. In view of the well recognized association in man of diabetes mellitus and another autoimmune endocrinopathy, chronic thyroiditis (Hashimoto's thyroiditis), the present studies were carried out to determine whether lymphocytic thyroiditis occurred with increased frequency in the diabetic, insulin-treated BB/W rat. The incidence of lymphocytic thyroiditis was strikingly increased in 8–10-mo-old diabetic rats (59%) as compared with their nondiabetic cohorts (11%) ($P < 0.001$). Relative thyroid weight was significantly greater in diabetic as compared with nondiabetic rats ($P < 0.01$) and in diabetic rats with thyroiditis than in diabetic rats without thyroiditis ($P < 0.025$). Lymphocytic thyroiditis was not accompanied by any consistent changes in serum T_4, T_3, and TSH concentrations or in the serum TSH response to thyrotropin-releasing hormone (TRH) suggesting that the thyroiditis was not of sufficient severity or duration to induce primary thyroid gland failure. The BB/W rat represents the first animal model of multiple autoimmune endocrinopathies and provides a unique opportunity to study the pathogenesis of these disorders. **DIABETES *30*:1058–1061, December 1981.**

The Bio Breeding/Worcester (BB/W) rat develops spontaneous insulin-dependent diabetes mellitus. Lymphocytic infiltration and destruction of the pancreatic beta-cells occurs in approximately 30% of BB/W rats between 60 and 120 days of age.[1,2] Since antiserum to rat lymphocytes attenuated or prevented the diabetes in affected or susceptible rats, respectively,[3] and neonatal thymectomy markedly reduced the frequency of diabetes,[4] a thymus-dependent cell-mediated autoimmune pathogenesis of the BB/W diabetic syndrome has been postulated. An autoimmune basis for the occurrence of spontaneous lymphocytic thyroiditis in the aged Buffalo rat,[5,6] Obese strain chicken,[7] White Leghorn pullet,[8] and Beagle dog,[9,10] has also been suggested. Since there is a well recognized association of insulin-dependent (type I) diabetes and lymphocytic thyroiditis in man, the present studies were carried out to determine whether spontaneous lymphocytic thyroiditis (LT) occurs in diabetic BB/W rats.

METHODS

Eight to ten-month-old insulin-dependent diabetic and nondiabetic male and female rats were obtained from the BB/W colony maintained by the Department of Pathology at the University of Massachusetts Medical School.

In the first experiment, 14 diabetic rats (8 males weighing 350–450 g and 6 females weighing 180–300 g) and 19 nondiabetic rats (9 males weighing 450–550 g and 10 females weighing 230–330 g) were studied. Rats were housed in hooded plastic cages and fed Purina Rat Chow and tap water ad libitum for 4 wk. Rooms were illuminated from 0700–1800 h and maintained at 20 ± 1°C. Diabetic rats were weighed and tested for glycosuria (Tes-Tape, Eli Lilly and Co., Indianapolis, Indiana) and ketonuria (Ketostix, Ames Division, Miles Laboratories, Inc., Elhart, Indiana) between 1300–1400 h daily. Treatment consisted of daily s.c. insulin (U-40 PZI, Eli Lilly and Co.), bicarbonate (8.4% Sodium Bicarbonate Inj., USP, Abbott Laboratories, North Chicago, Illinois), and fluids (Lactated Ringer's injection, USP, Travenol Laboratories, Inc., Deerfield, Illinois) as required to prevent dehydration, ketosis, and weight loss. Diabetic rats were permitted to have glycosuria so as to avoid hypoglycemic deaths. In general, the diabetic rats ate well, were

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active, and did not appear ill. At the time of killing, all rats were weighed and blood was obtained by decapitation without the use of anesthesia. Thyroid glands, which were carefully avoided during decapitation, were dissected free of connective tissue, weighed, and then placed in Bouin's fixative.

In the second experiment, 40 diabetic rats (20 males weighing 300–420 g and 20 females weighing 200–300 g) and 19 nondiabetic rats (10 males weighing 400–489 g and 9 females weighing 200–320 g) were maintained under the same conditions as described in the first experiment. All rats were weighed and anesthetized with urethane i.p. (120 mg/100 g body wt.). Synthetic TRH (50 ng/100 g body wt., Protirelin, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, New Jersey) was injected through a femoral vein catheter immediately after a baseline blood sample had been obtained. Ten minutes later, blood was obtained by decapitation and the thyroid glands were removed and fixed as described above.

Histologic sections of the thyroid glands were prepared and stained with hematoxylin and eosin. Sections from each thyroid were interpreted by one of us (A.A.L.) without knowledge of the presence or absence of diabetes mellitus. The extent of lymphocytic infiltration and follicle disruption was graded 0 to 4+, 0 indicating normal thryoid morphology and 1+ less than 10%, 2+ approximately 10–30%, 3+ approximately 30–50%, and 4+ greater than 50% histologic alterations. All sera from each experiment were quickly separated and stored at −20°C until assayed for hormone concentrations in duplicate and in the same assay. Serum T_4 and T_3 concentrations were measured by radioimmunoassay (RIA). Serum TSH concentration was measured by RIA with materials kindly supplied by the Hormone Distribution Officer, NIAMDD.

All values are expressed as the mean ± SE. Statistical analyses were carried out by the Student's and paired *t* tests and χ^2 analysis.

RESULTS

Morphologic findings. The histologic data from both experiments were combined, since the findings were similar. There were no sex-related differences in histology and results for males and females were, therefore, combined. Lymphocytic thyroiditis (LT) was observed in 11% (4/36) of the nondiabetic rats (range 1–2+, mean 1.5+) and in 59% (30/51) of the insulin-dependent diabetic rats (range 1–4+, mean 1.9+) ($\chi^2 = 23.1$, $P < 0.001$) (Table 1). Thyroid weight, expressed as mg thyroid/100 g body wt., was significantly greater in the diabetic as compared with the nondiabetic rats (10.0 ± 0.5 mg/100 g body wt. vs. 8.0 ± 0.5, $P < 0.01$). A positive correlation was observed between thyroid weight and the range and intensity of LT in the diabetic rats ($r = 0.43$, $P < 0.01$). Thyroid weight was significantly greater in diabetic rats with LT than in diabetic rats without LT (10.9 ± 0.7 vs. 8.7 ± 0.5, $P < 0.025$). Histologic sections of the adrenal glands from diabetic and nondiabetic rats revealed no evidence of lymphocytic infiltration.

TABLE 1
Incidence and severity of lymphocytic thyroiditis in the BB/W rat*

	Severity of thyroiditis					Incidence of thyroiditis
	0	1+	2+	3+	4+	(%)
Diabetic (N = 54)	21	12	11	6	1	59
Nondiabetic (N = 38)	32	2	2	0	0	11

* The histologic preservation was inadequate for critical interpretation in 3 diabetic and 2 nondiabetic animals.

Thyroid function studies. In the first experiment, there was no significant difference in the serum T_4, T_3, and TSH concentrations between diabetic and nondiabetic rats (Table 2). In the diabetic animals, serum T_4, T_3, and TSH concentrations were not significantly different in rats with or without thyroiditis (T_4, 4.7 ± 0.7 μg/dl vs. 4.3 ± 0.4; T_3, 86.1 ± 14.6 ng/dl vs. 74.0 ± 9.6; TSH,* 66.0 ± 20.9 μU/ml vs. 36.2 ± 3.5; respectively). There was, however, a marginally significant positive correlation between the intensity of thyroid lymphocytic infiltration and serum TSH concentration in the diabetic rats ($r = 0.58$, $P < 0.05$).

In the second experiment, basal serum T_4 and TSH concentrations were also not significantly different in diabetic and nondiabetic rats (Table 2). In the diabetic rats, the presence of LT did not affect the basal serum T_4 concentration; the serum TSH was slightly lower in rats with LT (43.9 ± 5.8 vs. 76.4 ± 14.5, $P < 0.05$). There was no correlation be-

* Readers should be aware that TSH values in the rat are normally higher than those found in human plasma.

TABLE 2
Thyroid function tests in BB/W rat

	Serum T_4 (μg/dl)	Serum T_3 (ng/dl)	Basal serum TSH (μU/ml)	TRH stimulated serum TSH (μU/ml)	ΔSerum TSH† (μU/ml)
Experiment 1					
Diabetic (N = 14)	4.5 ± 0.4	80.1 ± 8.6	50.0 ± 10.3	—	—
Nondiabetic (N = 19)	4.2 ± 0.2	89.1 ± 6.4	79.4 ± 13.6	—	—
Experiment 2					
Diabetic (N = 40)	3.7 ± 0.2	50.8 ± 2.1*	55.5 ± 6.7	351.0 ± 26.6*	295.4 ± 24.7‡
Nondiabetic (N = 19)	3.7 ± 0.4	51.8 ± 1.7	50.1 ± 5.7	446.6 ± 42.1	396.5 ± 41.0

Values are means ± SE.
* Blood obtained 10 min after the i.v. injection of 50 ng TRH/100 g body wt.
† Difference between basal and TRH stimulated serum TSH concentrations.
‡ $P < 0.05$.

tween the intensity of LT and basal serum TSH concentrations in the diabetic rats. Ten minutes after the administration of TRH, the serum T_3 and TSH concentrations were similar in diabetic and nondiabetic rats (Table 2), but the increment in the serum TSH concentration (difference between basal and 10-min values) was marginally lower in diabetic rats (295 ± 24.7 vs. 397 ± 41.0, $P < 0.05$). There was no relationship between the TRH-induced increment in serum TSH concentration in diabetic rats and the presence or extent of LT.

DISCUSSION

Diabetic BB/W rats were found to have a much higher incidence of spontaneous LT than their nondiabetic cohorts (59% vs. 11%). This is consistent with previous findings in man demonstrating an association between insulin-dependent diabetes and autoimmune thyroid disease, including the presence of circulating antithyroid antibodies,[11–14] lymphocytic or Hashimoto's thyroiditis,[15] and Graves' disease.[16] While spontaneous LT has been described in other species,[5–10] the present observations represent the first description of LT occurring in an animal with coexisting autoimmune diabetes mellitus. Although there is a marked female predominance in autoimmune thyroid disease in man,[16] LT occurred with equal frequency in male and female diabetic BB/W rats. This was not unexpected, however, since there is no sex-related difference in the frequency or severity of diabetes in the BB/W rat[17] or in the frequency of spontaneous LT in other species.[5–10]

In spite of the presence of LT in the diabetic rats, there was no conclusive evidence of hypothyroidism since serum T_4, T_3, and TSH concentrations and the serum TSH response to TRH were not consistently abnormal as compared with both nondiabetic BB/W rats and insulin-treated diabetic rats without thyroiditis. The variable degree of follicular disruption might explain the apparent absence of significant thyroid functional failure. Early thyroid failure as evidenced by an elevated serum TSH concentration or an enhanced TSH response to TRH in patients with Hashimoto's thyroiditis is often not observed until late in the course of this disease. Perhaps hypothyroidism would have been observed if older diabetic rats with LT had been evaluated. Further studies will be carried out to determine the age at which LT appears in the diabetic BB/W rats.

The etiology of the diabetes and thyroiditis is, at present, unclear. Studies in the BB/W rat indicate that both neonatal thymectomy and the administration of antiserum to rat lymphocytes ameliorate or prevent the occurrence of diabetes, supporting the hypothesis of a thymus-dependent cell-mediated autoimmune pathogenesis for this disorder.[3,4] Neonatal thymectomy has also been reported to reduce the frequency of experimentally induced LT in the guinea pig[18] and White Leghorn chicken.[19] In contrast to these observations, neonatal thymectomy in the Obese strain chicken increased[20] and neonatal bursectomy decreased[21] the severity of spontaneous thyroiditis while treatment with antilymphocyte serum had no effect on the severity of the thyroiditis.[22] In the outbred Wistar rat, T-cell depleted produced by thymectomy shortly after weaning followed by whole body irradiation resulted in the occurrence of LT and serum antithyroglobulin antibodies in approximately 60% of treated rats.[23] The differing effect of T-cell depletion on the evolution of thyroiditis in these species suggests that the helper or suppressor role of the T-lymphocyte is genetically determined. Thus, the inherent hyperactivity of the B-lymphocyte in the Obese strain chicken with spontaneous thyroiditis is enhanced by neonatal thymectomy while in the outbred Wistar rat, T-lymphocyte suppression (thymectomy plus irradiation) is responsible for the initiation of de novo thyroiditis. Although the presence of an intact thymus appears to be essential for the full expression of the diabetic syndrome in BB/W rats,[4] the effect of neonatal thymectomy on the frequency of LT has not yet been determined. Thus, the role of the T-lymphocyte in the pathogenesis of spontaneous thyroiditis in the BB/W rat is not yet known.

The major histocompatibility (B) genes in the Obese strain chicken have been shown to influence the incidence of LT and antithyroglobulin antibodies.[24] This observation suggests that immune response genes at, or closely linked to, the major histocompatibility locus may produce spontaneous thyroiditis via control of the T-lymphocyte.

The present studies demonstrate that spontaneous LT frequently occurs in diabetic BB/W rats, strongly suggesting that the animals that develop autoimmune insulitis also evidence autoimmune thyroiditis. The low incidence of thyroiditis in the nondiabetic BB/W rats also suggests a close association between these two autoimmune disorders. This conclusion is further supported by preliminary data that antithyroglobulin antibodies are more frequent in diabetic than in nondiabetic BB/W rats.

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