Autoantibodies in the BB/W Rat

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SUMMARY

The BioBreeding/Worcester (BB/W) rat is a model of spontaneous autoimmune diabetes mellitus and lymphocytic thyroiditis. Additional features supporting an immunologic pathogenesis of the BB/W syndrome include the protective action of antilymphocyte serum, neonatal bone marrow transfusions, and neonatal thymectomy. To evaluate other manifestations of immune dysregulation, the BB/W colony was surveyed for the presence of autoantibodies to a variety of tissue and cell constituents. Anti-smooth muscle and anti-thyroid colloid antibodies were present with great frequency in diabetic animals as well as in normoglycemic offspring of diabetic parents. Anti-parietal cell antibodies were less frequent and islet cell cytoplasmic and adrenal antibodies were not detected. These data suggest that the underlying defect in the BB/W rat is more likely to be an abnormal immune regulatory system than antigenically altered target tissues ("altered self") under attack by a normal immune surveillance system. DIABETES *31*:816–820, September 1982.

Diabetes mellitus occurs spontaneously in approximately 40% of a partially inbred colony of BioBreeding/Worcester (BB/W) rats. The salient features of the syndrome include the genetic predisposition for the development of diabetes;[1] the abrupt onset of insulin-deficient, ketosis-prone diabetes between 60 and 120 days of age; lymphocytic insulitis with rapid and virtually complete destruction of pancreatic beta-cells;[2] and lymphocytic thyroiditis in many diabetic and normoglycemic rats without detectable abnormalities of thyroid function.[3] Other immunologic features of the syndrome include the observations that (1) injections of antiserum to rat lymphocytes prevent and ameliorate the diabetic syndrome;[4] (2) neonatal thymectomy prevents diabetes;[5] (3) neonatal bone marrow transfusions reduce the frequency of diabetes;[6] and (4) susceptibility to develop diabetes is linked to the major histocompatibility complex.[7,8]

The frequent association of diabetes and thyroiditis in the BB/W rat, and the observation that BB rats obtained from the Canadian government colony evidenced autoantibodies to several tissues and subcellular constituents,[9] prompted us to evaluate the BB/W colony of the University of Massachusetts for the presence of autoantibodies. These studies were deemed desirable at this time for the following reasons: (1) it would allow us to test the hypothesis that BB/W rats evidence a more generalized abnormality of their immune regulatory apparatus than the specific attack by a normal immune system against "altered self" (i.e., pancreatic beta-cells and thyroid follicular cells); and (2) if BB/W rats do synthesize autoantibodies to a variety of cell and tissue components, it would be useful to obtain baseline data at the present stage of colony inbreeding for future comparisons when congeneic animals have been developed.

MATERIALS AND METHODS

Animals. The initial studies were performed on sera obtained from: (1) Acute and chronic diabetic animals from our breeding facility that were 3–8 mo of age and required daily insulin injections for survival. (2) Four-month-old phenotypically normal but genotypically diabetic rats [nondiabetic offspring produced by matings of diabetic (*dd*) × diabetic (*dd*) parents]. According to our genetic data,[1] these rats are homozygous for the diabetes gene, but remain phenotypically normal because of presently unknown genetic and environmental factors that prevent the expression of the diabetes gene. (3) Animals produced from two family lines with low or no incidence of diabetes (control rats).

After the preliminary experiments indicated that autoantibodies to smooth muscle and thyroid colloid were very frequent, the study was enlarged to include representatives of

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seven lines of rats that were in the ninth or tenth generation of brother × sister matings in the University of Massachusetts colony. Five of the lines are considered to be homozygous for the diabetes gene, because they have been through several *dd* × *dd* matings. All litters produced by breeders from these lines evidence diabetes with a frequency of at least 40–50%. Also included were the two lines of control animals: the W line has produced no diabetics in five generations of inbreeding (N = 142); the V line has produced two diabetics in 66 offspring over three generations.

Sera. Blood samples were obtained from animals at the time of death (left ventricular exsanguination or decapitation), or from the orbital venous plexus or tip of tail of rats not killed. Animals ranging in age from 1 day to 8 mo were sampled. Sera were separated, stored at −65°C, and diluted 1:10 with phosphate-buffered saline (PBS) before use.

Substrate. The thyroid lobes of nondiabetic BB/W rats were frozen in an IEC cryostat. Six- to eight-micron frozen sections were "fixed" in −20°C absolute acetone and used within 1–2 h, or stored in −20°C absolute acetone for a maximum of 24 h. Sections of thyroid were used for most of the reported studies. Selected sera were also incubated with identically processed frozen sections of normal BB/W rat kidney for the evaluation of smooth muscle antibodies and with normal BB/W pancreas, adrenal, and stomach, for the presence of islet cell cytoplasmic, adrenal, and parietal cell antibodies.

Reagents. Fluorescein-conjugated rabbit anti-rat IgG was purchased (Cappel Laboratories, Cochranville, Pennsylvania), reconstituted, and stored at −65°C. Before use it was absorbed with mouse liver powder (10 mg/ml), centrifuged, and diluted 1:50 with PBS.

Immunofluorescent technique. Indirect immunofluorescent staining was performed as follows: cryostat sections were rinsed and hydrated in several baths of PBS, followed by sequential incubations with the test sera and fluorescein-conjugated anti-rat IgG in moist chambers at room temperature, for 40 min each. Sections were rinsed for 30 min, with three changes of PBS after each incubation period. After the final rinse, sections were mounted in buffered glycerin and examined with a Zeiss Photomicroscope equipped for epi-illumination with a 50-W mercury lamp. The sections were coded and examined (A.A.L.) without knowledge of the source of serum sample. Two control slides were included with each group of test sera: (1) the initial incubation with rat serum was omitted, and (2) a serum sample previously read as negative was used for the initial incubation.

In the initial study, sera were evaluated for the presence of antibodies to smooth muscle (ASM), thyroid colloid (ATC), and islet cell cytoplasm (ICA). Subsequently, several sera with strongly positive ASM and ATC fluorescence were incubated with cryostat sections of adrenal, kidney, and stomach to evaluate further the ASM antibody and to test for the presence of adrenal and parietal cell antibodies. In the study of the seven family lines being inbred in our colony, only ASM and ATC were evaluated. The staining intensity of each sample was arbitrarily graded 0–5+ for each constituent. A staining index was calculated for each group of animals by totaling the assigned numerical grade (1–5+) for each rat and dividing by the number of positive sera in each group.

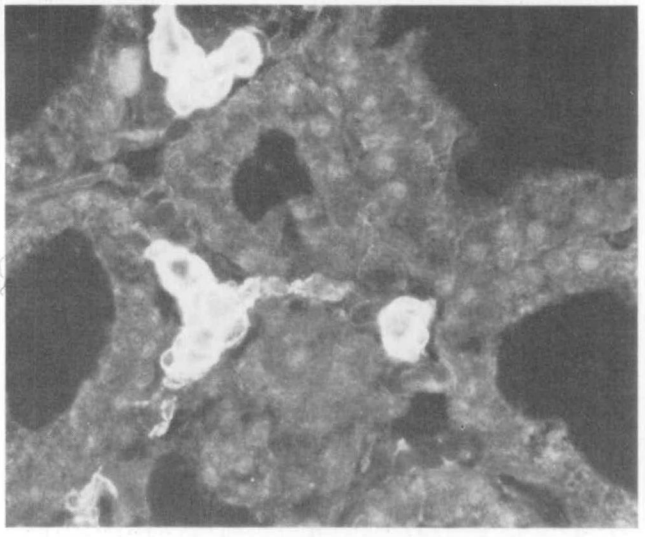

FIGURE 1. Anti-smooth muscle antibody staining is illustrated in this cryostat section of a nondiabetic BB/W thyroid. Brightly fluorescent arterioles are located between follicular epithelial cells that surround follicle lumina containing nonfluorescent colloid.(×350.)

RESULTS

Diabetic rats frequently evidenced autoantibodies to smooth muscle (Figure 1), thyroid colloid (Figure 2), and a variety of other cellular constituents. Since the most consistent autoantibodies detected were those directed against smooth muscle (ASM) and thyroid colloid (ATC), only these data are presented. The frequency, pattern, and intensity of autoantibodies among male and female rats were indistinguishable, and these data have been combined.

Table 1 illustrates the results of the initial study of sera obtained from the adult control, diabetic, and nondiabetic animals. Note that the combined frequency of ASM and ATC antibodies among phenotypically diabetic rats and phenotypically normal but genotypically diabetic was essentially

FIGURE 2. Intense anticolloid antibody staining is localized to the follicle lumina of this cryostat section of thyroid. The apparent fluorescence of follicle cell cytoplasm is a reflection of section thickness rather than of the presence of specific antimicrosomal antibodies. (×224.)

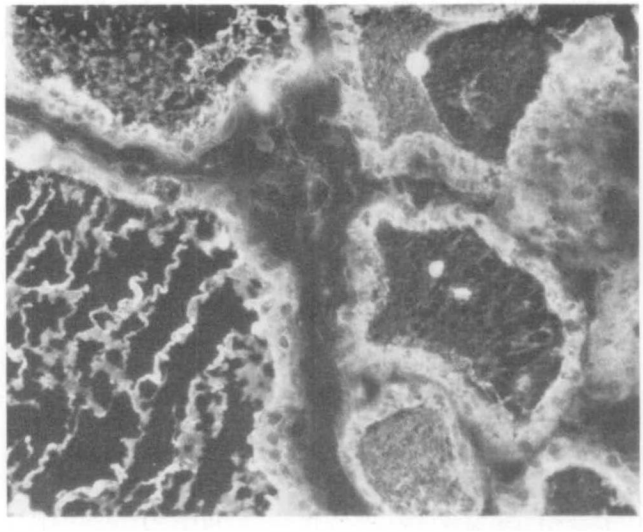

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TABLE 1
Autoantibodies in BB/W rats

	Control*	Diabetic†	Nondiabetic‡
Smooth muscle	1/40 (2.5%)	59/75 (79%)	45/50 (90%)
Thyroid colloid	12/40 (30%)	61/84 (73%)	37/50 (74%)
Combined frequency§	13/40 (32.5%)	77/84 (92%)	48/50 (96%)
Islet cell (cytoplasmic)	0/21	0/48	0/19

* Rats from family lines evidencing no or infrequent diabetes (see text).
† Acute and chronic diabetics combined.
‡ Nondiabetic offspring of *dd* × *dd* matings (genotypic diabetics).
§ Rats with either anti-smooth muscle or thyroid colloid antibodies or both.

the same (92% and 96%, respectively). Animals were included under "combined frequency" if their sera were positive for either, or both, ASM and ATC antibodies. In contrast, the frequency of ASM, ATC, and combined ASM/ATC antibodies among controls was significantly lower. All of the sera tested were negative for islet cell cytoplasmic antibodies (ICA).

Among the 84 diabetic sera tested, samples from 10 rats were selected that evidenced strongly positive ASM and ATC antibodies. When tested with adrenal and stomach sections, none of these samples revealed evidence of anti-adrenal antibodies and two (20%) revealed anti-parietal cell antibodies. When these and other sera strongly positive for ASM were incubated with sections of kidney, only the smooth muscle components of arteries, arterioles, and veins evidenced fluorescence. Glomeruli (including mesangial elements), proximal and distal tubules, and peritubular fibrils were consistently negative (Figure 3). Sera positive for ASM uniformly stained smooth muscle elements of all tissues tested, such as pancreas, stomach, adrenal, kidney, and thyroid. Although many serum samples were positive for both ASM and ATC, others were discordant for these autoantibodies (Figure 1) (data not illustrated).

FIGURE 3. Anti-smooth muscle staining is limited to the small artery and arteriole illustrated in this micrograph. The renal glomerulus and peritubular fibrils are negative. (×224.)

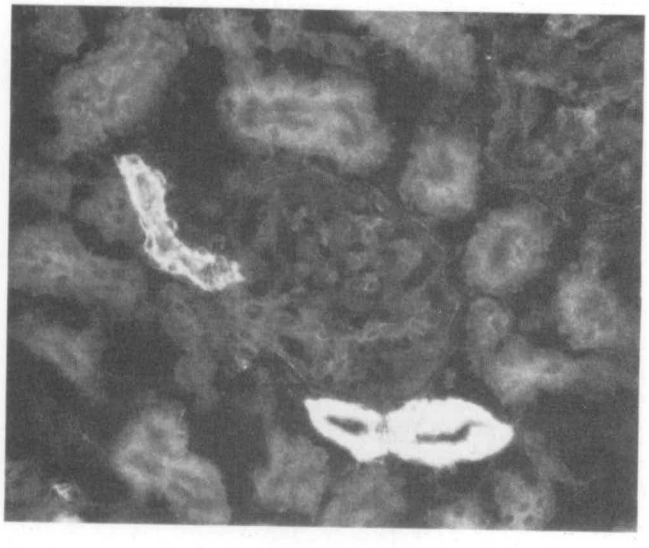

TABLE 2
Autoantibodies in BB/W rats (Nondiabetics)

	Colloid	Staining index	Smooth muscle	Staining index
Genotypic diabetics				
10 days	7/7	2.8	7/7	4.3
20–23 days	17/21	1.5	17/21	1.4
31 days	0/9		8/9	1.9
35 days	0/8		5/8	1.1
41 days	0/10		0/10	
45 days	2/9	1.5	4/9	1.1
50 days	0/10		3/10	1.3
60 days	3/9	1.0	7/9	2.1
70 days	6/7	2.0	7/7	2.1
80/120 days	42/55	2.1	50/55	2.6
Control rats				
V line (120 days)	3/17	1.0	0/17	
W line (120 days)	9/23	1.0	1/23	1.0

The presence of lymphocytic thyroiditis was determined in 31 of 45 diabetic rats evaluated for autoantibodies. Fifteen of twenty-one animals (75%) evidenced both thyroiditis and ATC. In contrast, 5 of 10 diabetic animals (50%) without thyroiditis evidenced ATC.

Table 2 illustrates the frequency of ASM and ATC antibodies in sera obtained from the seven lines (family groups) presently being inbred in the BB/W colony. All animals were sampled at the ninth or tenth generation of inbreeding and were normoglycemic at the time sera were obtained. Animals labeled "genotypic diabetics" have been through more than one *dd* × *dd* mating and are considered homozygous for the diabetes gene.[1] Control rats include animals with a low (V line) or absent (W line) frequency of diabetes in 3–5 generations of sib-matings.

Among normoglycemic, genotypic diabetic animals, either ASM or ATC or both antibodies were almost universally present, with a high staining intensity, at 10 days of age. At 20–23 days, both ATC and ASM were somewhat less frequent, with a decreased staining intensity. Subsequently, ATC antibodies disappeared until 45 days, while ASM-staining intensity decreased and finally disappeared at 41 days. Both ATC and ASM antibodies reappeared at 45–60 days and evidenced increasing staining intensity and frequency as the animals approached 120 days of age.

Nondiabetic control V and W lines evidenced ASM and ATC antibodies with very low frequency and low staining intensity at 120 days of age.

DISCUSSION

Although the precise pathogenesis and etiology of diabetes in the BB/W rat is not yet firmly established, the accumulated experimental data strongly suggest that these unique animals evidence abnormal immune regulatory mechanism(s). The initial abnormality that led to the animal's discovery, the spontaneous destruction of pancreatic beta-cells with resulting insulin-dependent diabetes, is preceded by lymphocytic insulitis.[2] It has since been reported that antiserum to rat lymphocytes will prevent and ameliorate the diabetic syndrome.[4] More recently, neonatal thymectomy has been shown to prevent, almost universally, the diabetic syndrome.[5] The results of both experimental interventions strengthen the (auto)immune pathogenesis of the

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syndrome and implicate the key role of thymus-derived/regulated cytotoxic T-cells. Neither the neonatal thymectomy, the antilymphocyte serum experiments, or the recent reports that susceptible BB rats evidence lymphopenia before, as well as after, the onset of diabetes[10,11] provide definitive insight regarding the magnitude of the immune regulatory abnormalities.

The data presented here suggest that BB/W rats also evidence immune regulatory defects that are manifested by the synthesis of antibodies directed against a variety of cellular and tissue constituents. Although BB/W rats also evidence autoantibodies of the IgG class that bind with skeletal muscle, nuclear protein, and gastric parietal cells, the data presented above concern predominantly anti-smooth muscle, anti-thyroid colloid, and anti-islet cell antibodies. We could find no evidence of anti-adrenal antibodies, and only 20% of a small number of sera evidenced gastric parietal cell antibodies. The data indicate that a large majority of diabetic rats synthesize ASM and ATC. The results also reveal that phenotypically normal but genotypically diabetic rats produce ASM and ATC at an early age (45–60 days), before the mean age at detection of hyperglycemia (92.4 days[1]). These data suggest that more than one cell type or tissue and possibly more than one antigenic determinant is under immunologic scrutiny and/or has been subjected to lymphocytic attack. These results also suggest that the underlying defect in the BB/W rat is likely to be an abnormal immune regulatory system rather than antigenically altered target tissues or cells (i.e., "altered self"), recognized by a normal immune surveillance system. This concept carries important theoretical as well as practical significance and increases the similarity of the BB rat to the human patient with diabetes and other polyendocrine abnormalities.

The data presented in this communication do not address, in detail, the relationship of the autoantibodies and pathology. Although this issue is best left to future studies addressed to specific organ systems, the following summary statements are appropriate:

(1) There are no presently recognized vascular lesions in diabetic or nondiabetic BB/W rats that might be related to the vascular smooth muscle binding of ASM antibodies. Smooth muscle lesions are also not recognized in the muscularis mucosa or muscularis propria of the gastrointestinal tract that also bind ASM antibodies. Perhaps the detection of these lesions will depend on long-term studies of older BB/W rats, or on the ability to detect more subtle, nonmorphologic abnormalities.

(2) Although there is high concordance between the presence of ATC and lymphocytic thyroiditis among diabetic rats, 50% of a small group of animals without thyroiditis also evidenced ATC. It would appear likely, therefore, that the synthesis of ATC is not dependent on the presence of lymphocytic thyroiditis.

(3) The provocative absence of ICA in animals with demonstrated islet pathology and with high titers of other autoantibodies is unexplained at present. In prior studies (data not presented), sera from animals with glucose intolerance and low-grade insulitis were also negative for ICA. These rats may be important because they include examples of long-term persistent beta-cell injury, which, it might be argued, would be more likely to induce ICA than the typical BB diabetic rat, wherein beta-cell destruction is rapidly consummated. Among the 48 diabetic serum samples examined in the present study, ICA were sought but not detected even in samples that were strongly positive for both ASM and ATC. Using a radioligand protein-A technique, Dyrberg et al.[13] reported the possible presence of islet cell surface and splenic lymphocyte antibodies in 85% of BB rats shortly after the detection of diabetes. The significance of this interesting report will be clarified by a detailed description of the natural history of these antibodies in diabetic and nondiabetic animals and strengthened by early confirmation of these findings by another laboratory.

(4) The presence of antibodies in normoglycemic but genotypically diabetic rats and their absence, or low frequency in the two nondiabetic control lines, suggest that the presence of ATC and ASM may be useful as a marker for the diabetes gene. This possibility will receive close scrutiny as inbreeding of all family lines continues.

(5) The high frequency and staining intensity of ATC and ASM in neonatal rats is probably due to the transfer of IgG from autoantibody-positive mothers to nursing newborn pups via the colostrum. This explanation will be examined critically after cesarian section delivery and foster parenting to lactating, control-line antibody-negative females.

The data presented in this article strengthen our understanding of the autoimmune syndrome in the BB/W rat. In addition to the presence of lymphocytic insulitis and thyroiditis, the BB/W rat is now recognized as capable of synthesizing a variety of autoantibodies that appears to be unrelated to specific tissue pathology. In this regard, the BB/W rat can be distinguished from the several mouse models of autoimmunity,[14] wherein the presence of autoantibodies to thymocytes and red blood cells and the trapping of antigen-antibody deposits within renal glomeruli are usually associated with well-defined immunologic diseases. The BB/W rat is more appropriately compared with patients with viral-induced autoantibody production (infectious mononucleosis and infectious hepatitis) and with the multimammate mouse, *Praomys (Mastomys) natalensis,* a rodent intermediate in size between the mouse and the rat. *Praomys* do not become diabetic and infrequently reveal thyroiditis, but do synthesize antibodies to smooth muscle, thyroid colloid, skeletal muscle, and nuclear antigens, all in the absence of recognized pathologic alterations.[15]

Finally, it should be re-emphasized that although the pathogenetic significance of autoantibodies in BB/W rats is unclear, they are not always associated with diabetes or thyroiditis. The data presented above suggest that BB/W rats have extraordinarily mature (i.e., antibody-synthesizing) B-lymphocytes. The mechanism(s) responsible for T- and B-cell dysfunction with resulting diabetes, thyroiditis, and autoantibody production is not clear and future studies are required to clarify the manifestations of the immune regulatory defect(s) as well as its etiology and pathogenesis.

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Note added in proof. After this article was accepted for publication, Elder et al.[16] reported the presence of autoantibodies to gastric parietal cells, smooth muscle, and thyroid colloid antigens in BB rats obtained from Ottawa, Canada and the University of Massachusetts colony.

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