Restriction Fragment Polymorphisms in the Major Histocompatibility Complex of Diabetic BB Rats

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SUMMARY

DNA isolated from diabetic BB (BB/Hagedorn) rats was examined for restriction fragment length differences within the major histocompatibility complex (MHC) as compared with nondiabetic (W-subline) BB rats. Polymorphisms were detected using a mouse class I MHC gene as probe. Specifically, a 2-kb BamHI fragment was present in all the nondiabetic rats examined, but absent in the diabetic rats. Similar polymorphisms were observed with various other restriction enzymes, particularly XbaI, HindII, and SacI. There were no polymorphisms detected using either a human DR-α (class II antigen heavy chain) or a human DC-β (class II antigen light chain) gene as probes. These results indicate that the BB rat diabetic syndrome may be linked to differences in class I MHC genes.

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The BB rat spontaneously develops an insulin-dependent diabetes (IDDM) with many characteristics similar to those found in human IDDM (see ref. 1). The disease appears on a background of severe immune deficiency[2] and is associated with insulitis[3] and islet cell surface antibodies.[4] These and other immunologic aspects of the disease suggest that the BB rat suffers from an autoimmune disorder with possible involvement of the major histocompatibility complex (MHC). Indeed, genetic studies have established that the disease is linked to the rat MHC with a requirement for the $RT1^u$ haplotype.[5,6] However, the complexity of the mammalian MHC precludes a detailed examination of all MHC gene products that may play a role in the development of the disease because many of the genes are undetectable by conventional serologic or immunologic techniques. Conversely, examination of restriction fragment length polymorphism in genomic DNA using the hybridization of cloned DNA probes overcomes this problem and allows rapid screening of whole families of genes. As a study preliminary to a more detailed genetic examination of the BB rat MHC, we have examined the diabetic BB rat for restriction fragment polymorphisms in both class I and class II MHC genes as compared with a closely related but nondiabetic control line of BB rats.

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MATERIALS AND METHODS

The diabetic BB line used in these studies originated from the BA line at the University of Massachusetts, Worcester[7] and was a gift of Dr. David MacLennan (University of Toronto). The rats are maintained in strict brother-sister breeding in our animal facility and are designated as strain BB/Hagedorn (BB/H). In the 16–18th generations of inbreeding from which animals were obtained, the prevalence of diabetes was 85% at 120 days of age. The nondiabetic control line (BB-W subline) originated from Worcester, Massachusetts, and was a gift of Dr. Arthur Like. The W-subline was originally derived from the BA line at the fifth generation of brother-sister mating[7] and has since been bred separately. Diabetes has not been observed during the 16 generations of inbreeding. Animals in the 16th generation had normal levels of lymphocytes and no signs of diabetes by 120 days of age. All animals used in this study have been followed since birth by a number of parameters including urine glucose, blood glucose, islet cell surface antibodies, and blood lymphocyte count up to the day of death (about 120 days of age).

Nuclei were prepared from the livers of freshly killed animals using standard procedures.[8] Nuclei preparations were then digested overnight at 37°C with 0.25 mg/ml proteinase K in 10 mM Tris-HCl (pH 7.4), 12 mM EDTA, and 10 mM NaCl. After phenol-chloroform extraction, the DNA was precipitated with ethanol and resuspended in 10 mM Tris-HCl (pH 8.0) containing 1 mM EDTA.

Restriction enzymes were purchased from New England Biolabs (Beverly, Massachusetts) or Boehringer-Mannheim (Indianapolis, Indiana) and used according to the suppliers'

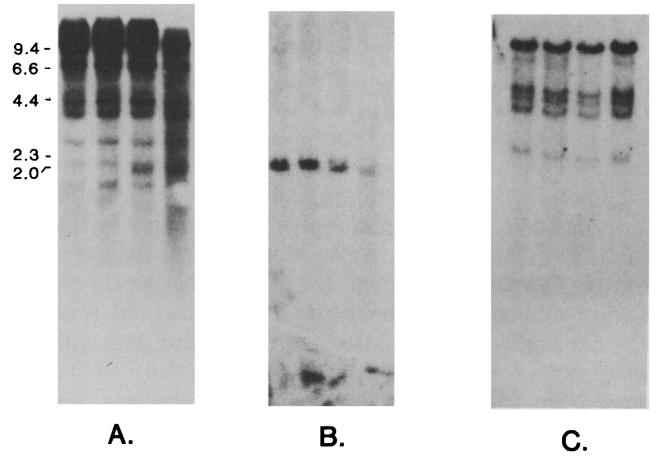

FIGURE 1. Restriction fragment polymorphisms as detected by Southern blotting of diabetic and nondiabetic control DNA. Molecular weight marker was a HindIII digest of λ-DNA, and the weights appear to the left of panel A. The pair of lanes to the left in each panel are each from a different diabetic rat (BB/H strain) while the pair to the right are from the nondiabetic control strain. (A) BamHI-digested DNA probed with ^{32}P-pH2^{d}-1 DNA. (B) HindII-digested DNA probed with ^{32}P-pHLA-DRα1 DNA. (C) BamHI-digested DNA probed with ^{32}P-pHLA-DCβ1 DNA.

recommendations. Digested DNA preparations were loaded onto 1% agarose gels in 30 mM Tris-acetate (pH 7.2) with 1 mM EDTA and subjected to electrophoresis. The DNA in the gels was transferred to nitrocellulose filters by the method of Southern.[9] After transfer, the filters were hybridized overnight at 42°C with ^{32}P-nick translated cloned DNA probes in 50% formamide, 0.75 M NaCl, 0.075 M sodium citrate, 5× Denhardt's solution, 50 mM sodium phosphate (pH 6.5), and 0.1 mg/ml denatured salmon sperm DNA. After hybridization, the filters were washed four times at 52°C in 15 mM NaCl, 1.5 mM sodium titrate, and 0.1% SDS, dried, and subjected to autoradiography. The probes used in this study were mouse pH2d-1 (class I),[10] human HLA-DRα (class II, α-chain)[11] and human HLA-DCβ (class II, β-chain).[12] All were cDNA clones and gifts of Dr. P. A. Peterson (Uppsala, Sweden).

RESULTS

Total genomic DNA was prepared from rat liver and digested with various restriction enzymes. After agarose gel electrophoresis, the DNA was transferred to nitrocellulose filters and hybridized with the radioactive DNA probes. When a cloned cDNA representing a mouse class I MHC gene was used as hybridization probe, the banding pattern was quite complicated with each enzyme tested, reflecting the complexity and number of class I genes in the rat. We were able to detect differences between the restriction patterns of the diabetic BB/Hagedorn rats compared with the control line. After digestion with BamHI, a band at approximately 2 kb was present in the BB-control animals, but was absent in the diabetic BB animals (Figure 1A). In subsequent studies involving animals from different generations, this band was present in each of six control animals and absent in all of seven diabetic animals examined (data not shown). In each of 11 restriction enzymes used, including the enzymes XbaI, HindII, and SacI, restriction fragment length polymorphism between diabetic and nondiabetic BB rats was detected at the class I loci.

In a similar manner, possible restriction fragment polymorphism in the rat class II MHC genes was studied using either a human HLA-DR α-chain cDNA clone or a human HLA-DC β-chain clone as probe. In both instances, we were unable to detect polymorphisms between diabetic and control animals using either BamHI digestion of the DNA (Figure 1B,C) or digestion by 10 other restriction enzymes (Bg1I, EcoRV, EcoRI, HindII, HindIII, PstI, PvuII, SacI, Sa1I, and XbaI) (data not shown).

DISCUSSION

These studies demonstrate that within the MHC of the rat, there are marked differences between diabetic and nondiabetic BB rats. These differences appear as polymorphisms detected by screening restriction digests of total genomic DNA using a cloned mouse class I MHC gene as a probe. The polymorphisms were observed to be stably inherited among several rats spanning two generations. In contrast, we were unable to detect a difference within the class II MHC genes of these two BB rat lines. In a recent study, polymorphism at the MHC class II locus was reported between high- and low-incidence BB rats.[13]

However, it is of critical importance in this respect that the nondiabetic control BB rats are as closely related to the diabetic animals as possible. The further removed the control group is from the diabetic, the more chance there is for finding polymorphisms that do not involve the diabetic syndrome. The diabetic BB line (BB/Hagedorn) used in these studies is currently in its 18th generation of inbreeding, the incidence of diabetes being 85% at 120 days of age in the 16th generation. The control line (designated "W") was derived from the same diabetic line at the 5th generation of brother-sister mating[7] and has produced no diabetic animals after 16 generations of inbreeding. The control BB (W-subline) rat is currently free of hyperglycemia, glucosuria, insulitis, and lymphopenia, but may have islet cell surface autoantibodies that are detected at an increased frequency in prediabetic and diabetic BB/Hagedorn rats.[14] Despite these differences it is important to emphasize that all BB rats share the same MHC haplotype as determined by serologic typing.[5]

These results differ from recent observations in human IDDM where polymorphisms were detected[15] using the same HLA-DC-β-chain probe employed here. In these studies, specific polymorphisms were detected at class II-light chain loci between HLA-DR identical diabetic and nondiabetic individuals. Since we were unable to detect class II MHC polymorphisms associated with diabetes in the BB rat, it is possible that the genetic lesion resulting in the disease may be different in the rat. Alternately, we cannot exclude the possibility that the HLA-DC-β-probe was not sufficiently homologous to regions of the rat class II loci, which may contain a polymorphism.

It is impossible at this point to specify which class I MHC gene contains the polymorphism(s) observed here. All class I genes, both the transplantation antigens as well as the differentiation antigens (Qa and T1a in the mouse) share considerable homology. Since the two BB rat lines are identical at the RT1^{u} locus, it is tempting to infer that the polymorphism is not a phenomenon at that locus. However, the polymorphic region may be a silent one occurring within the

gene encoding the $RT1^u$ haplotype detected by serologic methods. The gain or loss of a restriction enzyme site may occur after any mutational event, including point mutations, translocations within or outside coding sequences for a class I heavy chain gene. However, the observation of additional polymorphisms with several other restriction enzymes, with completely different recognition sites, rules out a simple point mutation, and suggests a more extensive genetic event such as a deletion or translocation. Resolution of these questions awaits the isolation and characterization of the gene(s) involved.

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