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# Molecular characterization of MHC class II antigens ($\beta_1$ domain) in the BB diabetes-prone and -resistant rat

**Nelson J. Chao, Luika Timmerman, Hugh O. McDevitt, and Chaim O. Jacob**

Department of Microbiology and Immunology, Stanford University, Stanford, CA 94305, USA

**Abstract.** The BB or BB/Worcester (BB/W) rat is widely recognized as a model for human insulin-dependent diabetes mellitus (IDDM). Of at least three genes implicated in genetic susceptibility to IDDM in this strain, one is clearly linked to the major histocompatibility complex (MHC). In an attempt to define the diabetogenic gene(s) linked to the MHC of the BB rat, cDNA clones encoding the class II MHC gene products of the BB diabetes-prone and diabetes-resistant sublines have been isolated and sequenced. For comparison, the $\beta_1$ domain of class II genes of the Lewis rat ($RT1^L$) were sequenced. Analysis of the sequence data reveals that the first domain of RT1.$D_\beta$ and RT1.$B_\beta$ chain of the BB rat are different from other rat or mouse class II sequences. However, these sequences were identical in both the BB diabetes-prone and BB diabetes-resistant sublines. The significance of these findings is discussed in relation to MHC class II sequence data in IDDM patients and in the nonobese diabetic (NOD) mouse strain.

## Introduction

A large body of evidence now indicates that type I diabetes of man, the BB (Bio-Breeding) rat, and the NOD (Nonobese Diabetic) mouse results from autoimmune beta cell destruction within the islets of Langerhans of the pancreas. The beta cells are progressively destroyed, resulting in insulin deficiency and clinically apparent hyperglycemia (Nakhooda et al. 1977). Both the BB rat and the NOD mouse manifest several immune abnormalities (Makino et al. 1980) including morphologic evidence of early infiltration of the islets by mononuclear cells and detection of anti-islet cell antibodies (Baekkeskov et al. 1984) remarkably similar to insulin-dependent diabetes mellitus (IDDM) in humans (Lernmark et al. 1985).

*Offprint requests to:* C. O. Jacob

A strong association with class II major histocompatibility complex (MHC) genotype has been reported in the human (Nerup et al. 1984) and in both rodent models of autoimmune diabetes (Colle et al. 1981, 1986a, Jackson et al. 1984, Hattori et al. 1986, and Wicker et al. 1987). As in human IDDM, which has a high frequency of *HLA-DR3* and *DR4* alleles, the rat disease is controlled in part by the genes encoding the *RT1* (the rat MHC) region.

Genetic studies crossing BB diabetes-prone rats with non-diabetes-prone rat strains have shown that only rats expressing the $RT1^u$ haplotype develop IDDM (Colle et al. 1981). Furthermore, only the class II subregion of the $RT1^u$ complex appears to be necessary for disease expression (Colle et al. 1986a). Both BB diabetes-prone (BBS) rats and BB resistant control lines (BBN) are homozygous for $RT1^u$ haplotypes. Moreover, because no recombinant MHC haplotypes separating the $B^u$ and $D^u$ class II loci are currently available, genetic studies cannot establish the exact MHC subregion determining the expression of IDDM in BB rats.

To further define the inheritance of the histocompatibility region of the BB rat in relation to the development of diabetes, we undertook to clone and sequence class II MHC genes of the BB diabetes-prone rat and compared it with its diabetes-resistant sister strain, the BB resistant rat, derived from the original colony of outbred Wister rats which gave rise to the BB rat.

## Materials and methods

*Rats.* The BB diabetes-prone (BBS) strain and its sister strain, the BB-diabetes resistant (BBN) strain, were kindly provided by Drs. Like and Guberski of the University of Massachusetts. Both rat lines were derived from the same parents and were in the 25–28th generation of brother-sister mating.

*cDNA Libraries.* Complementary DNA (cDNA) libraries were prepared in λgt10 by a modification of a described procedure (Acha-Orbea et al. 1987). The libraries were screened with $A^k_\beta$ and $E^s_\beta$ probes (Estess et al. 1986) at a density of 10 000 plaque-forming units per 150 mm plate.

Sequence was determined using the dideoxy chain termination procedure modified for use with $^{35}$S-labeled dATP (Amersham, Arlington Heights, Illinois). Sequences were determined on both strands using M13mp18 as vector. At least two clones were isolated and sequenced on both strands for each allele using universal primers and synthetic oligonucleotide produced from existing sequences.

*Polymerase chain reaction.* The polymerase chain reaction (PCR) was performed by a modification of the procedure described by Saiki and co-workers (1986). Total RNA was used and cDNA was prepared as described (Acha-Orbea et al. 1987). Two oligonucleotides (18 mers) were used, one with a perfect match and the second with four base pair mismatches at the 5′ end. Thirty cycles were carried out. This allowed the amplification of a ~200 bp fragment which encoded the region from amino acid 24–83. The fragment was cloned onto M13mp18 vector and sequenced.

## Results

*Nucleotide sequence determination of the first external domain of RT1.B$_\beta$.* The first external domain of the RT1.B$_\beta$ chain of the BB rat is different from previously sequenced rat or mouse class II sequences (Fig. 1). However, this sequence was identical in both the BB diabetes-prone and BBN rats.

In comparison with RT1.B$^b_\beta$ (Figueroa et al. 1988), there are differences scattered throughout the molecule. There is 85% similarity between the two sequences at the nucleotide level and 73% similarity in the amino acid sequence. Interstingly, in comparison with the NOD mouse A$_\beta$ sequence (Acha-Orbea et al. 1987), there is even greater similarity (88% at the nucleotide level and 83% in the amino acid sequence). This is surprising in that one would expect greater homology with the *RT1.B* allele of another rat than with that of a mouse equivalent.

We therefore felt it necessary to determine the nucleotide sequence of the *RT1.B$_\beta$* allele from a different rat strain. Because most of the variability among class II alleles is confined to the first extracellular domain (Todd et al. 1988), we used the polymerase chain reaction for rapid in vitro DNA amplification to produce large quantities of first-domain fragment, which was then gel purified and directly cloned into the M13 sequencing vector. We used cDNA synthesized from total RNA of a Lewis rat (RT1$^L$) spleen as substrate for amplification. Interestingly, the RT1.B$_\beta$ partial nucleotide sequence of the first domain obtained from the Lewis rat was different by only four base pairs from the respective sequence in the BB rat, resulting in three amino acid differences at positions 34, 50, and 70.

*Nucleotide sequence determination of the first external domain of RT1.D$_\beta$.* The first domain sequence of RT1.D$_\beta$ of the BB rat is given in Figure 2. Again, there was no difference between the sequences of the BBS and the BBN sublines.

No previous RT1.D$_\beta$ chain sequences are available for comparison. Because of this, the polymerase chain

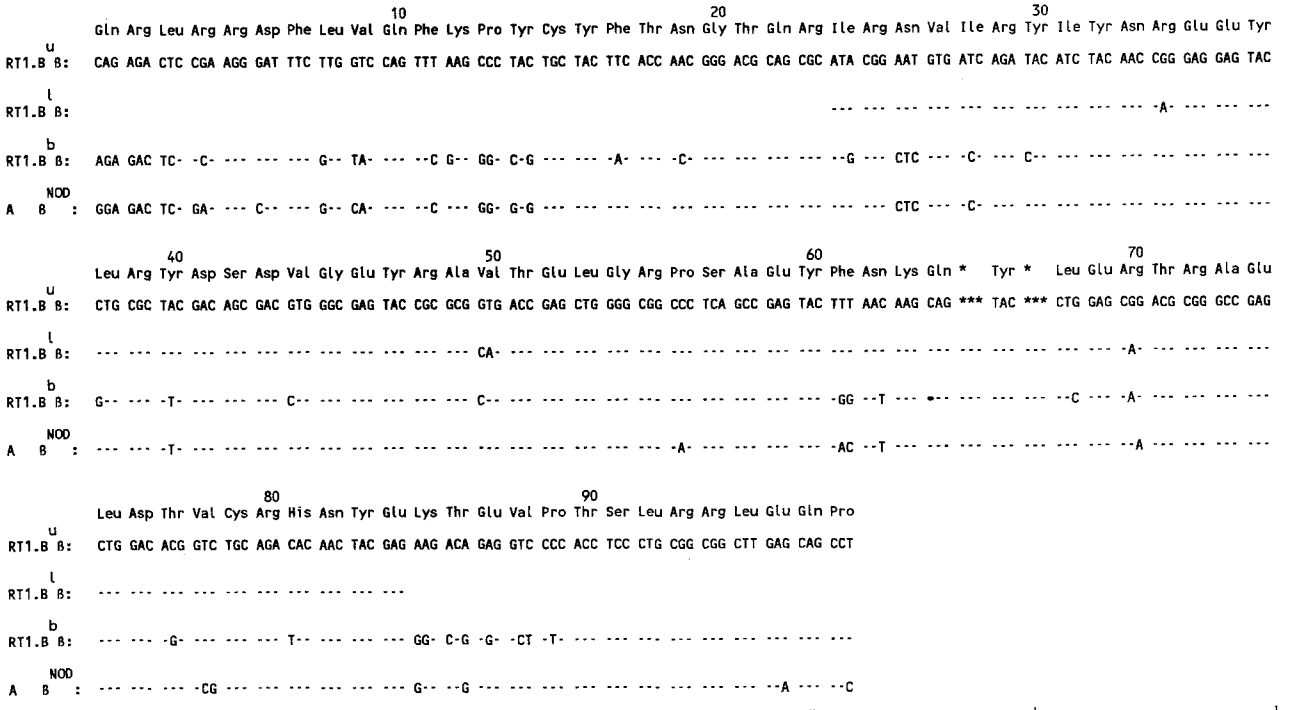


**Fig. 1.** Comparison of the cDNA nucleotide sequence of the first external domain of RT1.B$^u_\beta$ from the BB rat, RT1.B$^b_\beta$ from the BUF rat, RT1.B$^l_\beta$ from the Lewis rat, and the A$_\beta$ chain from the NOD mouse. *Above* the sequences, the amino acid sequence of the BB RT1.B$^u_\beta$ chain is indicated. *Asterisk** indicates deletions introduced to line-up sequences

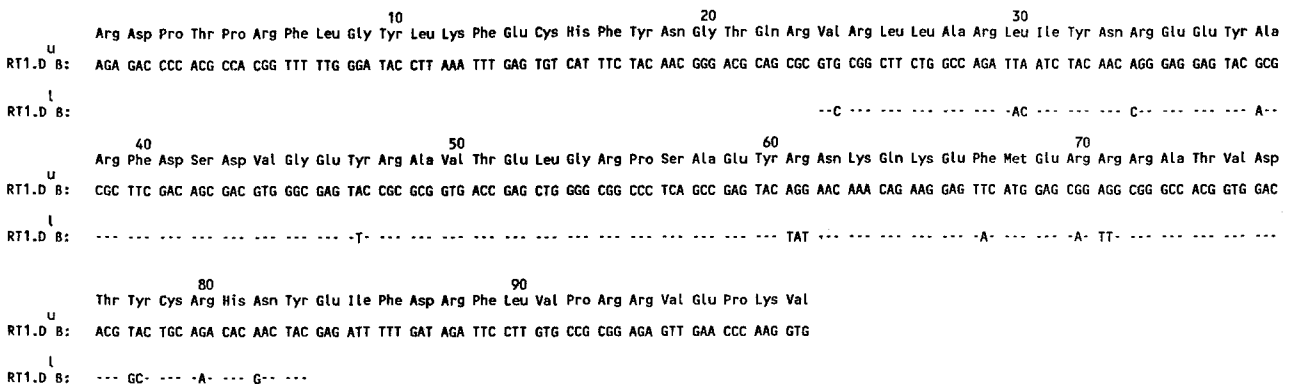


**Fig. 2.** Comparison of the cDNA nucleotide sequence of RT1.$D_\beta^u$ of the BB rat and RT1.$D_\beta^l$ from the Lewis rat. *Above* the sequences, the predicted amino acid sequence of the BB RT1.$D_\beta^u$ chain is indicated

reaction was used for cloning and partial sequencing of the Lewis rat RT1.$D_\beta$ chain. As shown in Figure 2, there are 17 base pair differences between the two sequences scattered throughout the first domain, between codons 24 and 83.

It was shown previously (Todd et al. 1987) that comparison of DQB chains commonly found in human diabetics have either Ala, Val, or Ser at position 57, while DQB chains rarely found in IDDM patients have Asp at this position.

When examining the BB rat RT1.$D_\beta$ and RT1.$B_\beta$ sequences around amino acid 57 in the first domain, both alleles have serine at this position (not Asp), which is consistent in principle with the above observation. However, both sublines of the BB rat possess exactly the same sequence.

For further comparison, we have partially sequenced the first domain of the RT1.$B_\beta$ and RT1.$D_\beta$ chain from the Lewis rat ($RT1^L$). This strain of rat was chosen since crosses between the BB rat and Lewis rat have shown that diabetes developed only in rats that possessed the *RT1*$^u$ haplotype from the BB parent (Colle et al. 1986a). The region around amino acid 57 in the first domain of both the RT1.$B_\beta$ and RT1.$D_\beta$ chains of the Lewis rat are identical to those of the BB rat. Moreover, the BUF rat ($RT1^b$) has a serine at position 57 as well, and not aspartic acid, as it would be predicted from the model (Figueroa et al. 1988).

## Discussion

Structural analysis of MHC class II genes in IDDM patients indicates that *DQB* alleles that have a noncharged amino acid at position 57 of the first domain are significantly enriched in Caucasian IDDM populations. On the other hand, possession of Asp 57-positive *DQB* alleles provides resistance to IDDM (Todd et al. 1987). This observed correlation is further supported by inspection of the $A_\beta$ sequence isolated from the NOD mouse (Acha-Orbea et al. 1987). The first domain sequence of the NOD mouse $A_\beta$ chain, which is homologous to the human DQB chain, is unique among mouse $A_\beta$ alleles in that it has a Ser residue at position 57. All other published mouse $A_\beta$ alleles have Asp at position 57 (Estess et al. 1986). These data would suggest that the NOD $A_\beta$ gene contributes to disease susceptibility. We show here that in the BB rat, class II $\beta$ chains from both IDDM-sensitive and -resistant strains have identical first-domain sequences with Ser at position 57. Moreover, the region around amino acid 57 in class II $\beta$ chains from the nondiabetic Lewis rat are identical to those in the BB rat. The BUF-RT1.$B_\beta$ chain also has a Ser at position 57 (Figueroa et al. 1988). Thus, the data presented here suggest that the allelic form of amino acid 57 is not a disease susceptibility marker in the rat model of autoimmune diabetes.

As for the lack of differences between the two sublines of BB rats in the $B_1$ domain of class II genes, it is possible that differences in the $B_2$ domain, the transmembrane, or intracytoplasmatic region are playing a role in the development of the disease, although this hypothesis is unlikely. A second hypothesis, involving nucleotide sequence differences in the $\alpha$ chains of these molecules as reponsible for diabetic susceptibility in the BB rat, was not tested in this report. However, the absence of differences between the two sublines of the BB rat in their class II $\beta$ chain sequences does not mean that these genes themselves are not involved in the pathogenesis of the disease. Indeed, it was shown recently that the BB-resistant subline can develop insulitis, although this will not progress to overt diabetes as in the BB-prone subline (Greiner et al. 1987). Similarly, in the NOD model of diabetes it was shown that the MHC-linked diabetogenic gene is not required for the development of insulitis (Wicker et al. 1987).

Since susceptibility to diabetes is multigenic (Colle et al. 1986b, Prochazka et al. 1987), it is equally plausible

that the difference between the sublines is confined to a gene outside the MHC. Alternatively, differences in expression of MHC class II genes or their inducibility by lymphokines [as was shown in the genetic susceptibility to experimental allergic encephalomyelitis development in mouse and rat (Massa et al. 1987)] may be relevant for diabetic susceptibility in the BB model.

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