Identification of a Major Gene Responsible for Type 1 Diabetes in the Komeda Diabetes-Prone Rat

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Abstract: Type 1 diabetes mellitus is an autoimmune disease involving both environmental and genetic factors. Genetic analyses in humans and rodents have shown that the major histocompatibility complex (MHC) is a major genetic factor and that several other genes may be involved in the development of the disease. We performed genetic analysis of type 1 diabetes in a newly established animal model, the Komeda diabetes-prone (KDP) rat, and found that most of the genetic predisposition to diabetes is accounted for by two major susceptibility genes, MHC and Iddm/kdp1. In addition, we identified a nonsense mutation in the Casitas B-lineage lymphoma b (Cblb) gene by positional cloning of Iddm/kdp1. In this paper, I review our positional cloning analysis of Iddm/kdp1 and propose a two-gene model of the development of type 1 diabetes in which two major susceptibility genes, Cblb and MHC, determine autoimmune reaction and tissue specificity to pancreatic β -cells, respectively.

Key words: autoimmune disease, Cblb gene, Komeda diabetes-prone rat, major histocompatibility complex, type 1 diabetes

Introduction

Most type 1 diabetes mellitus has autoimmune pathogenesis by which insulin secreting pancreatic β -cells are destroyed by the patient's own lymphocytes. While both environmental and genetic factors are involved, the major histocompatibility complex (MHC) has been identified as the only major genetic factor, although several other genes are thought to be minor factors [4].

Several spontaneous animal models of type 1 diabetes have been developed and genetically analyzed. In the non-obese diabetic (NOD) mouse, more than eigh-

teen loci have been reported, and the MHC was identified as the only major factor [14]. In the BioBreeding (BB) rat, at least six loci have been mapped, and two major loci, MHC and *Lyp*, are involved. A frameshift mutation in the immune-associated nucleotide 4 like 1 (*Ian4ll*) gene has been identified in *Lyp* [7, 12]. Although the genes of most other responsible loci have not yet been identified, these findings suggest that different combinations of genes may contribute to the development of diabetes in several animal models.

The Komeda diabetes-prone (KDP) rat is a newly established animal model in type 1 diabetes research

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[10]. We performed genetic analysis of type 1 diabetes in the KDP rat, and found that most of the genetic predisposition to diabetes could be accounted for by two major susceptibility genes, MHC and *Iddm/kdp1* [15]. We also identified a nonsense mutation in the Casitas B-lineage lymphoma b (*Cblb*) gene by positional cloning of *Iddm/kdp1* [16].

In this paper, I review our positional cloning analysis of *Iddm/kdp1* and propose a two-gene model of the development of type 1 diabetes in the KDP rat.

Komeda diabetes-prone (KDP) rats

The KDP rat was established by Komeda and colleagues [10] as a diabetes-prone substrain of the Long-Evans Tokushima Lean (LETL) rat [8]. The phenotypic features of the KDP rat are autoimmune destruction of the pancreatic β -cells, rapid onset of overt diabetes with no sex difference, and lack of significant T-cell lymphopenia. Thus it resembles human type 1 diabetes. Most of the animals show moderate to severe lymphocyte infiltration into pancreatic islets (insulitis), and about 80% develop diabetes within 220 days of age [10]. However, the maintenance and production of KDP rats for use in research has been a continuing problem due to the poor reproductive ability of the animals. To solve this, we recently established the KDP rat as a segregating inbred strain having the *Iddm/kdp1* region in the heterozygous state [17]. About 80% of *Iddm/kdp1* homozygous rats develop diabetes with severe insulitis, while neither heterozygous nor wild-type rats develop diabetes.

Genetic analysis of type 1 diabetes in the KDP rat

Inheritance of type 1 diabetes

Genetic analysis of the LETL rat shows that a gene closely linked with the MHC is involved in insulitis [8], suggesting involvement of the MHC in the diabetes of the KDP rat. Accordingly, we used Tester Moriyama (TM) and Long-Evans Tokushima Otsuka (LETO) rats of the same MHC haplotype as KDP rats, and Brown Norway (BN) rats of a different haplotype to generate three backcrosses: (TM $\times$ KDP) F1 $\times$ KDP, (LETO $\times$ KDP) F1 $\times$ KDP, and (BN $\times$ KDP) F1 $\times$ KDP (TM cross, LETO cross, and BN cross) [15].

Diabetes was not observed in F1 but was observed in N2 rats. The cumulative frequency of diabetes in N2 rats varied with the cross: at 120 days of age, 32 and 34% of the animals in the TM and LETO crosses developed diabetes, respectively, while 16% of the animals in the BN cross developed diabetes. The cumulative frequency of diabetes in the TM and LETO crosses was about half that of KDP, while that of the BN cross was about one fourth, suggesting that the inheritance of diabetes might be explained by segregation of a single recessive gene in the TM and LETO crosses and by segregation of two such genes in the BN cross. If that was the case, then one of the two genes should be the MHC.

Genome wide scan for type 1 diabetes susceptibility genes

We first performed a genome wide scan using the TM cross, and successfully mapped one recessive gene, *Iddm/kdp1*, on a 9 cM genomic segment between *Mox2* and *D11M16Mit46* on rat chromosome 11 [15]. We genotyped N2 rats generated from LETO and BN crosses and found strong evidence of linkage to diabetes in the same region on chromosome 11, confirming the recessive locus *Iddm/kdp1*. In addition, we also found evidence of linkage to diabetes in the MHC region on chromosome 20 in the BN cross. These results demonstrate that *Iddm/kdp1* is a major susceptibility gene for type 1 diabetes, and that most of the genetic predisposition to diabetes in these crosses is accounted for by the segregation of *Iddm/kdp1* and MHC [15].

Comparative mapping of the Iddm/kdp1 region in rat, mouse and human

We performed comparative mapping of the *Iddm/kdp1* region in rat, mouse and human, and demonstrated that orthologues of *Iddm/kdp1* are located in the region between *Gap43* and *D16Mit46* on mouse chromosome 16 and in the p11-q13 region on human chromosome 3 [15]. As susceptibility genes for type 1 diabetes have not been mapped on these chromosomal regions in human and mouse, *Iddm/kdp1* is believed to be a novel susceptibility gene for the disease.

Positional cloning of *Iddm/kdp1*

Fine genetic mapping of Iddm/kdp1

To clone *Iddm/kdp1* by positional cloning strategy,

we first constructed a precise genetic and comparative map of rat chromosome 11 and mouse chromosome 16 in the vicinity of the *Iddm/kdp1* locus [16]. Analysis of 65 diabetic N2 rats in the TM cross placed the locus in a genomic segment of 3.1 cM between *D11M16Mit14* and *D11Yok1*. The mouse intersubspecies backcross consisting of 1,655 progeny refined the orthologous segment to 3.0 cM between *D16Mit14* and *D16Mit46*, allowing construction of a physical map of the region in both species.

Physical mapping of Iddm/kdp1 and genome analysis

We constructed a physical map using YAC and BAC libraries of rat and mouse [16]. The *Iddm/kdp1* critical region is covered entirely by rat clones and partially by mouse clones. By sequencing these clones and by searching with BLAST, we identified two known genes, activated leukocyte cell adhesion molecule (*Alcam*) and *Cblb*, in the critical interval. ALCAM is a CD6 ligand, and CD6-ALCAM interaction may be involved in thymocyte-thymic epithelial cell adhesion and regulation of T-cell function [2], suggesting *Alcam* as a potential candidate for *Iddm/kdp1*. Sequence analysis of *Alcam* cDNA showed no obvious mutation except the missense variation Val184Leu, and RT-PCR analysis indicated that mRNA expression was not altered in KDP rats. However, the Val184Leu variation is not specific to KDP, as several rat strains have the same variation [17].

Identification of a nonsense mutation in Cblb gene

Cblb is another potential candidate for *Iddm/kdp1*, as *Cblb* deficient mice show several autoimmune phenotypes due to enhanced activation of T-cells [1, 3]. Sequence analysis of *Cblb* cDNA of KDP rats revealed a homozygous nonsense mutation Arg455X [16]. *Cblb* was identified as an isoform of proto-oncogene Cbl (c-Cbl) [9], and functions as a ubiquitin protein ligase [6] consisting of a tyrosine kinase binding domain, a ring finger domain, several proline rich regions, and a leucine zipper domain. The KDP mutation truncates the protein in the middle, deleting the proline rich regions and leucine zipper domain. The proline rich regions are required for binding of *Cblb* to its target molecules [6]. Accordingly, the mutation in KDP rats should be loss-of-function, which implies responsibility in the development of the disease in KDP rats.

Functional role of Cblb in T cells

Signaling through the T-cell receptor and co-stimulatory signaling through CD28 are both required for T-cell activation (See Fig. 1). In the resting state, *Cblb* ubiquitinates and inhibits PI3-kinase, an important molecule in CD28 signaling [6]. Upon stimulation, CD28 signaling inhibits *Cblb* [18], resulting in the activation of PI3-kinase and the T cells. Thus, *Cblb* is critical in the regulation of T-cell activation. *Cblb* deficient mice show abnormal T-cell activation in the absence of CD28 signaling, and exhibit several features of autoimmune disease, including lymphocyte infiltration into multiple tissues and production of autoantibody, but do not develop diabetes [1]. Thus, *Cblb* is an important regulator of autoimmunity, suggesting that dysregulation of the signaling pathway in which *Cblb* is involved may contribute to human autoimmune diseases including type 1 diabetes. However, disease causing mutations in *CBLB* have not been reported so far. Association studies of polymorphisms of *CBLB* in type 1 diabetes found that *CBLB* is not a major genetic factor in the disease in U.K. and U.S. multiplex type 1 diabetic families [11, 13]. The relationship of *CBLB* with other autoimmune diseases may be clarified by mutation screenings and association studies.

Histological analysis of the KDP rat

We also performed histological analysis of the KDP rat [16]. Severe lymphocyte infiltration into the thyroid gland and mild to moderate lymphocyte infiltration into various tissues in addition to the pancreatic islets was observed, including the submandibular gland, kidney, adrenal and pituitary. The histological findings are similar to those in *Cblb* deficient mice, excepting the severe insulitis in KDP rats.

Transgenic rescue experiment

To confirm responsibility of the *Cblb* mutation in the KDP phenotype, we carried out a transgenic rescue experiment [16]. Wild-type *Cblb* cDNA under regulation of mouse H-2Kd promoter was used as a transgene construct. H-2Kd promoter leads to ubiquitous expression of the transgene. We obtained two independent transgenic lines for the H-2Kd promoter. At 120 days of age, transgenic line 1 was completely rescued from the development of diabetes, and transgenic line 2 was rescued partially but significantly. These data show

Fig. 1. Two-gene model of the development of type 1 diabetes in the KDP rat. Both *Cblb* and MHC class II genes are involved in the development of diabetes in this model, the former as a critical regulator of T-cell activation and the latter in antigen presentation to T cells. The *Cblb* mutation leads to autoimmune reaction, and the MHC class II u haplotype confers tissue specificity to the pancreatic β -cells. In combination, these two states cause insulinitis and type 1 diabetes in the KDP rat.

clearly that *Cblb* is responsible in the development of diabetes in the KDP rat.

Model of development of type 1 diabetes in the KDP rat

We suggest a two-gene model of the development of type 1 diabetes in the KDP rat in which two major genes are involved in the development of the disease (Fig. 1). *Cblb* determines autoimmune reaction while the MHC confers tissue specificity to pancreatic β -cells. The combination of these two states readily causes insulinitis, and results in type 1 diabetes. The *Cblb* mutation brings about abnormal T-cell activation and lymphocyte infiltration to various tissues, which leads to autoimmune reaction. The MHC class II u haplotype of the KDP rat is frequently distributed in rat strains, and all of the spontaneous and interferon-alpha inducer poly IC-induced models of type 1 diabetes in rats have this haplotype [5]. These data suggest that this class II molecule has specific binding capacity to an unidentified β -cell autoantigen that activates β -cell reactive T-cells, thus conferring susceptibility to insulinitis and type 1 diabetes. The relationship between MHC class II haplotypes and tissue specificity in autoimmunity may be clarified using congenic strains having specific MHC class II haplotypes and the *Cblb* mutation.

Conclusion

We have shown that *Cblb* is a major susceptibility gene for type 1 diabetes in the KDP rat and that *Cblb* functions as a negative regulator of autoimmunity. We propose a two-gene model of the development of type 1 diabetes in which *Cblb*, in combination with the MHC, accounts for most of the genetic predisposition to the disease. It remains to be determined whether or not *CBLB* contributes to human autoimmune diseases.

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