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Genetic and functional association of the immune signaling molecule 4-1BB (CD137/TNFRSF9) with type 1 diabetes☆

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Abstract

Idd9.3, a locus that determines susceptibility to the autoimmune disease type 1 diabetes (T1D) in the nonobese diabetic (NOD) mouse, has been mapped to the distal region of chromosome 4. In the current report we reduce the size of the *Idd9.3* interval to 1.2 Mb containing 15 genes, including one encoding the immune signaling molecule, 4-1BB, which shows amino acid variation between diabetes sensitive and resistant strains. 4-1BB, a member of the TNF receptor superfamily expressed by a variety of immune cells, mediates growth and survival signals for T cells. Functional analyses demonstrate that purified T cells from NOD congenic mice with the C57BL/10 (B10) allele at *Idd9.3* produce more IL-2 and proliferate more vigorously in response to anti-CD3 plus immobilized 4-1BB ligand than T cells from NOD mice with the NOD allele at *Idd9.3*. In contrast, the response to anti-CD3 plus anti-CD28 costimulation was indistinguishable between the congenic strains, pinpointing the differences in NOD versus NOD.B10 *Idd9.3* T cell responses to the 4-1BB costimulatory pathway. These data provide evidence in support of *Idd9.3* as the locus encoding 4-1BB and suggest that the 4-1BB signaling pathway could have a primary function in the etiology of autoimmune disease.

Keywords: Nonobese diabetic mouse; Congenic; Gene mapping; T lymphocyte; Costimulation

1. Introduction

In a process that is controlled by multiple loci, known as insulin-dependent diabetes (*Idd*) genes, the NOD mouse spontaneously develops T1D.[3] On mouse chromosome 4, there are three distinct regions that have been shown to affect T1D, *Idd9.1*, *Idd9.2* and *Idd9.3*. An NOD mouse, congenic for all three *Idd9* sub-regions

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[3] Abbreviations used: NOD.B10 *Idd9.3* refers to NOD mice having B10 DNA introgressed at the *Idd9.3* locus on chromosome 4. T1D, type 1 diabetes.

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introgressed from the diabetes-resistant B10 strain, is highly protected from T1D (less than 5% of females develop T1D as compared to 80% in the NOD parental strain) [1]. A B10-derived resistance allele at *Idd9.3* alone provides approximately 50% protection from T1D [1]. Because the mouse genomic sequence was not available when the *Idd9.3* interval was originally characterized, the definition of the *Idd9.3* region was only detailed to a 2.0-cM genetic interval (which approximates to a 4.0-Mb physical distance) containing an uncharacterized number of genes. Several loci were examined as candidate genes that could be potentially localized to the *Idd9.3* interval, including those encoding three tumor-necrosis factor receptor superfamily (TNFRSF) members *41bb* (*Tnfrsf9*, *Cd137*), *Wsl1* (*Tnfrsf25*, *Dr3*), and OX-40 (*Tnfrsf4*). Whereas OX-40 was found to be located outside of *Idd9.3* [1], the gene encoding 4-1BB was included in the *Idd9.3* region. The NOD and B10 4-1BB molecules differ in three amino acid positions: (1) a leucine (B10) to proline (NOD) substitution in the cytoplasmic domain at position 211, due to a T (B10) to C (NOD) SNP in exon 6; (2) a valine (B10) to alanine (NOD) substitution at position 24 in the extracellular domain, due to a T (B10) to C (NOD) SNP in exon 1; and (3) the insertion of an alanine in NOD between amino acids 174 and 175, due to insertion of GCT in exon 5 [1]. The WSL1 gene could not be positioned since no sequence variation in the gene was found [1].

This study was initiated following the release of mouse genomic sequence for the *Idd9.3* region that enabled the fine-mapping of the interval and ascertainment of gene content. We now report that the *Idd9.3* interval is a 1.2-Mb region containing 15 genes, including the gene encoding the structurally variant 4-1BB molecule but not the TNFRSF member WSL1.

Both CD4 and CD8 T cells play a role in NOD pathogenesis [2] and 4-1BB is well established as having costimulatory function for CD4 and CD8 T cells, in vitro and in vivo [3,4]. Although in vitro costimulation assays clearly show a role for 4-1BB in augmenting CD4 and CD8 T cell function [5], the role of 4-1BB in vivo is more complex, with different models showing roles for 4-1BB in enhancing or ameliorating immune responses. For example, mice lacking 4-1BB or its ligand have decreased memory CD8 T cell responses to viruses [6–8]. However, T cells from 4-1BB^{-} mice also show hyperresponsiveness to anti-CD3 [8]. Agonistic antibodies to 4-1BB can enhance anti-viral and anti-tumor immunity as well as GVHD [9–16]. Paradoxically, the same antibodies used to enhance anti-tumor immunity can suppress humoral immunity and autoimmune disease [17–21]. Given the extensive evidence for a role for 4-1BB in modulating immune responses, we have focused our attention on the hypothesis that 4-1BB is encoded by *Idd9.3*. The data included in the current report support this hypothesis since mice with the B10 and NOD *Idd9.3* regions produce functionally distinct responses to stimulation with 4-1BBL.

2. Methods

2.1. Animals

Female NOD/MrkTacfBR mice were purchased from Taconic Farms, Inc. (Germantown, NY). The subcongenic lines of NOD.B10 *Idd9* mice, NOD.B10 *Idd9*R11 (R11, having B10 resistant alleles at *Idd9.2* and *Idd9.3*) and NOD.B10 *Idd9*R35 (R35, *Idd9.3* resistant allele only), were developed as previously described [1]. Since R11 and R35 respond identically to 4-1BB stimulation, both strains are termed NOD.B10 *Idd9.3* and are used interchangeably in the current study. The R11 and R35 congenic strains are available through the Emerging Models Program at Taconic Farms (Lines 1105 and 1106, respectively). All procedures were approved by the Animal Care Committee of the University of Toronto, following the guidelines of the Canadian council on animal care.

2.2. Cell lines, antibodies, and reagents

The following hybridomas and cell lines were obtained from the American Type Culture Collection (Rockville, MD): anti-mouse CD3 (145-2C11), anti-CD11c (N418), anti-B220 (RA3-6B2), anti-MAC-1 (TIB-128), anti-heat stable antigen (M1/69), anti-rat Ig κ chain (RG7/7.6H2), and the IL-2 dependent cell line CTLL. The anti-mouse CD28 hybridoma (37.51.1) was provided by Dr. J. Allison (University of California, Berkeley, CA). A recombinant baculovirus encoding a soluble form of 4-1BB ligand (s4-BBL) was used to produce s4-1BBL as previously described [22]. A 4-1BBL-specific monoclonal antibody (TKS-1) was used to affinity purify the s4-1BBL from the insect cell supernatants as described [5].

2.3. Identification of new microsatellite markers and genotyping

Mice were initially genotyped by PCR using primers for the previously published microsatellite markers. To map the recombination points more precisely within the R35 and R15 strains, further microsatellites were identified and characterized. Sequences were obtained from the Ensembl mouse genome sequence (http://www.ensembl.org). Repeat sequences were then identified using RepeatMasker 4-Apr-2000 version (http://ftp.genome.washington.edu/RM/RepeatMasker.html) modified by D. Beare and L. Smink, JDRF/WT Diabetes and Inflammation Laboratory, University of