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Distinct genetic control of autoimmune neuropathy and diabetes in the non-obese diabetic background☆

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ABSTRACT

The non-obese diabetic (NOD) mouse is susceptible to the development of autoimmune diabetes but also multiple other autoimmune diseases. Over twenty susceptibility loci linked to diabetes have been identified in NOD mice and progress has been made in the definition of candidate genes at many of these loci (termed *Idd* for insulin-dependent diabetes). The susceptibility to multiple autoimmune diseases in the NOD background is a unique opportunity to examine susceptibility genes that confer a general propensity for autoimmunity versus susceptibility genes that control individual autoimmune diseases. We previously showed that NOD mice deficient for the costimulatory molecule B7-2 (NOD-B7-2KO mice) were protected from diabetes but spontaneously developed an autoimmune peripheral neuropathy. Here, we took advantage of multiple NOD mouse strains congenic for *Idd* loci to test the role of these *Idd* loci the development of neuropathy and determine if B6 alleles at *Idd* loci that are protective for diabetes will also be for neuropathy. Thus, we generated NOD-B7-2KO strains congenic at *Idd* loci and examined the development of neuritis and clinical neuropathy. We found that the NOD-H-2^{g7} MHC region is necessary for development of neuropathy in NOD-B7-2KO mice. In contrast, other *Idd* loci that significantly protect from diabetes did not affect neuropathy when considered individually. However, we found potent genetic interactions of some *Idd* loci that provided almost complete protection from neuritis and clinical neuropathy. In addition, defective immunoregulation by Tregs could supersede protection by some, but not other, *Idd* loci in a tissue-specific manner in a model where neuropathy and diabetes occurred concomitantly. Thus, our study helps identify *Idd* loci that control tissue-specific disease or confer general susceptibility to autoimmunity, and brings insight to the Treg-dependence of autoimmune processes influenced by given *Idd* region in the NOD background.

1. Introduction

The non-obese diabetic (NOD) mouse strain is a prototypic murine model of type 1 diabetes that has served as an important tool for dissecting mechanisms involved in the breakdown of immune tolerance in autoimmune diabetes [1]. In addition to diabetes, NOD mice are prone to development of other autoimmune syndromes including: autoimmune sialadenitis [2], autoimmune thyroiditis [3], autoimmune peripheral polyneuropathy [4], prostatitis in male mice [5] and some features of non-organ-specific autoimmune disease such as hemolytic anemia and late-onset anti-nuclear antibodies, a systemic lupus erythematosus (SLE)-like disease if exposed to killed mycobacterium [5–7]. Autoimmune diseases in NOD mice share many similarities to the comparable human diseases, including the presence of organ-specific autoantibodies, autoreactive $CD4^+$ and $CD8^+$ T cells, and genomic synteny of susceptibility loci. Thus, the NOD mouse has been considered as a good model for other autoimmune diseases such as Sjogren's Syndrome, Guillain–Barre Syndrome and MS [4,8,9].

Autoimmune diabetes in the NOD mouse is polygenically controlled by as many as two dozen loci (termed *Idd* for insulin-dependent diabetes) distributed over 14 chromosomes [10–12]. NOD congenic strains have been developed that contain NOD genome at all loci except for one (or a few) "introgressed" allelic

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variants from a non-autoimmune mouse strain, usually C57BL/6 (B6) or C57BL/10 (B10) mice. These congenic models have been instrumental for the definition of genes and pathways that contribute to susceptibility to autoimmune diabetes. *Idd1*was one of the first loci to be identified in the NOD mouse and spans the major histocompatibility complex (MHC) region of the NOD mouse [13–17]. NOD mice harbor a unique MHC haplotype, termed H-2^{g7}, which is necessary for the development of diabetes and represents the highest genetic contributor to disease susceptibility [18]. The exact gene or regulatory elements that are responsible for the effects of other *Idd* loci on disease still await identification but candidate genes have been identified for several *Idd* loci that are strong contributors to diabetes [10,11]. Besides the MHC, *Idd3* on chromosome 3 disproportionately contributes to the development of disease. NOD mice congenic for the *Idd3* locus have a strongly reduced incidence of diabetes and candidate genes include IL-2 and IL-21 [19–24]. Other individual *Idd* loci alter the development of diabetes to various degrees but interactions of protective alleles can confer stronger protection against diabetes in NOD mice. For example, introgressed B6 alleles for *Idd*5 or *Idd*10/*Idd*18 are almost fully protective for insulitis and diabetes when combined with the B6 *Idd3* region but they have more modest effects in isolation [23,25–28].

We previously showed that NOD mice deficient for the costimulatory molecule B7-2 (NOD-B7-2KO mice) were protected from autoimmune diabetes and sialadenitis but spontaneously developed autoimmune peripheral polyneuropathy [4]. NOD-B7-2KO mice exhibit limb paralysis associated with severe demyelination in the peripheral nerves beginning at 20–25 wk of age, and the disease affected 80–100% of NOD-B7-2KO females and 30–40% males by 30–35 wk of age [4]. Peripheral neuropathy in NOD-B7-2KO mice is dependent on interferon-gamma (IFN-γ)-producing $CD4^+$ T cells that include autoreactive $CD4^+$ T cells specific for peripheral nerve antigens such as myelin protein zero (P0) [4,29,30]. Autoimmune peripheral polyneuropathy has also been described in NOD mice after disruption of various pathways involved in immune tolerance such as IL-2, PD-1 or the autoimmune regulator (Aire) and appears to have comparable immunopathogenic properties as the NOD-B7-2KO disease [31–33]. In particular, NOD mice partially deficient in Aire function develop peripheral neuropathy that is mediated by CD4+ T cells targeting myelin P0 and IFN-γ is required for disease to develop [33–35], similar to what has been observed in NOD-B7-2KO mice [4,29,30]. In contrast, peripheral polyneuropathy does not occur in mice deficient for B7-2, Aire or PD-1 on B6 or mixed B6-129 backgrounds. This suggested that development of autoimmune peripheral neuropathy on the NOD background may be related to its genetic propensity to autoimmunity. Additionally, T cell responses and autoAbs directed at Schwann cells surrounding the pancreatic islets of Langerhans have been detected in NOD mice, and it was suggested that the insulitis that precedes overt clinical diabetes may be, in part, directed against this peri-islet Schwann cell network. NOD mice have also been shown to be susceptible to autoimmunity targeting the central nervous system and can develop experimental autoimmune encephalomyelitis (EAE) [9,36]. Taken together, these data raised the possibility that common genes and pathways may be implicated in autoimmune pathologies targeting the pancreatic islets and the nervous system in the NOD mouse. In this study, we examined this question by focusing on loci previously shown to confer susceptibility to autoimmune diabetes in NOD mice by crossing NOD-B7-2KO to selected NOD congenic strains in order to compare the genetic control of autoimmune diabetes versus neuropathy. The studies showed that there is only a partial overlap in the genetic control of diabetes versus neuropathy in the NOD background.

2. Materials and methods

2.1. Mice

NOD-B7-2KO mice have been described previously [4]. NOD mice congenic for *Idd1* (H-2^{h4} and H-2^b), *Idd3*, *Idd5*, *Idd9*, and *Idd10*/*18* were obtained from Taconic. NOD mice congenic for *Idd4* [37] were generously provided by Qing-Sheng Mi (Henry Ford Immunology Program, Detroit, MI). NOD-B7-2KO mice were crossed to individual NOD-*Idd* congenic mice and F1 mice (heterozygous for B7-2 and *Idd* alleles) were intercrossed to generate NOD-B7-2KO-*Idd* congenic mice. The genotype for B7-2 was determined by PCR of tail DNA as described [4]. The genotype for *Idd1* was determined by flow cytometry of PBMCs isolated from the tail vein using mAbs against H-$2K^d$ and I-A^{g7} (NOD allele), H-$2K^k$ (H-2^{h4} allele) and H-$2K^b$ (H-2^b allele). The genotype for other *Idd* loci was determined by PCR using primer pairs specific for DNA segments including *Idd3* (ATGAGTTGGGAAGCTTGTGC and GTAAAGGCCAAGGGAAAAGG for marker D3nds36), *Idd4* (TAAGAACCTTCTGTAGTTATT and ACCTTAGTTAGAGTTGGTCTC for marker D11Nds1; TTTCATGACCCCTAATTTCCC and GTGGGTGTGCCTGTCAATC for marker D11Mit39), *Idd5* (TCTAGGTTCCTGGGATAGAATCC and ATAGAAGCAGACCCAGAAGCC for marker D1Mit74; TATTGTTTATGGAAATTGGACCC and CATCTCTGAAGGAAAAAGTGCA for marker D1Mit132), *Idd9* (TGGTCATGTGTGTCCATGC and ACTTCATGTAGCCAGGTGGG for D4Mit233; GACAAACCACATGTAATGTGTGG and CTGCCTGCAGGCTGTATGTA for marker D4Mit180), and *Idd10*/*18* (TAGACCAATCTTGGGAGTGTCC and GGAAAAGCATAAGAAACAACCG for marker D3Mit12; ATCTGAGCAATCCAGAGTTAGTCA and GCAACCT CTGCATGCATG for marker D3Mit300). Indicated mice were treated with 50 μg anti-B7-1 mAbs every other day for 14 days between 2 and 4 weeks of age. Neuropathy and diabetes were assessed weekly as previously described [4,29]. Only female mice were used except where indicated. All mice were housed in a specific pathogen-free facility at The University of California at San Francisco. All animal experiments were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco.

2.2. Histology

For histological analysis, tissues were fixed in formalin and embedded in paraffin. Multiple 5 μm sections were stained with hematoxylin and eosin. For scoring of sciatic nerve and thyroid gland infiltration, scores of 0, 1, 2 and 3 indicate no, mild, moderate and severe lymphocytic infiltration, respectively.

2.3. In vitro cytokine production

Single-cell suspensions were prepared from spleen and LN of indicated mice. DMEM-glutamax medium (Life Technologies, Gaithersburg, MD) supplemented with 5% heat-inactivated FCS (Summit Biotechnology, Ft. Collins, CO), 100 U/ml penicillin, 100 U/ml streptomycin, nonessential amino acids, 10 mM HEPES and 50 μM β-mercaptoethanol (all from Life Technologies) was used for cell culture. For primary stimulation, spleen and LN cells (2×10^5) were stimulated with anti-CD3 (0.1 or 1 μg/ml) alone or together with anti-CD28 (1 μg/ml) mAbs. For secondary stimulation, cells were stimulated with anti-CD3 (1 μg/ml) and anti-CD28 (1 μg/ml) mAbs for 7 days, with addition of 20 U/ml recombinant human IL-2 on day 3. On day 7, cells were restimulated with anti-CD3 with or without anti-CD28 mAbs as above. Supernatant was harvested from triplicate cultures after one (IL-2) or two (IFN-γ) days for both primary and secondary stimulation. Levels of cytokine were measured by commercial ELISA kits according to the manufacturer's recommendations (BD-PharMingen, San Diego, CA).