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# Insulin-dependent diabetes loci *Idd5* and *Idd9* increase sensitivity to experimental autoimmune encephalomyelitis

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**Abstract**

The spontaneous development of autoimmune diabetes in NOD mice suggests that they are unable to establish and maintain immunologic self-tolerance. Congenic NOD mice expressing B10-derived alleles are protected from pancreatic beta cell destruction and autoimmune diabetes. To determine if the B10 alleles in loci *Idd5* and *Idd9* could influence susceptibility to autoimmunity in other organs, we compared MOG35–55-induced EAE in NOD mice to that of diabetes-resistant NOD.B10.Idd5 and NOD.B10.Idd9 mice. Surprisingly, the severity and chronicity of EAE were enhanced in the diabetes-resistant congenic mice. Our findings indicate that some alleles may influence susceptibility to immune-mediated damage in an organ or tissue-specific fashion, and highlight the necessity of disease-specific investigations.


*Keywords:* Autoimmunity; Encephalomyelitis; Diabetes; NOD; MOG; Idd5; Idd9

## Introduction

Autoimmunity is a biological phenomenon where components of the adaptive immune response, antibodies and T cells, target molecules encoded by the host genome. Overt autoimmune disease is a clinical condition mediated by autoimmune mechanisms that directly or indirectly provoke tissue damage and bring about physiological consequences [1]. Surprisingly, benign autoimmunity may be quite common—existing without clinical manifestations [2]. On the other hand, destructive or pathogenic autoimmunity is far less prevalent and may be confined to a subset of genetically predisposed individuals. One of the prevailing characteristics of several inflammatory autoimmune diseases is organ-specificity. Despite the development and spread of immune responses to several organ-associated molecules, autoimmune diseases are usually characterized and diagnosed by pathology in particular organs or tissues, as in arthritis [3], multiple sclerosis [4], diabetes [5], and thyroiditis [6]. Systemic Lupus Erythematosis is a systemic autoimmune disease, which would not display this pattern of focused organ-specific autoimmunity [7].

The events that lead to the priming and activation of self-reactive lymphocytes are still unclear; however, the organ-specificity of the pathology suggests that immune responses to particular antigenic epitopes may selectively prompt the breakdown of immunological homeostasis. Certainly, microbial organisms have long been suspected of contributing to the priming of self-reactive lymphocytes via molecular mimicry [8] or through the release of self-antigens following pathogen-induced tissue damage. While the inciting microbe is unknown in most cases, there are examples where distinct infectious agents have clear associations with outbreaks of autoimmune disease [9]. The expression of unique MHC alleles is also linked to increased susceptibility and progression of autoimmune diseases such as, ankylosing spondylitis [10], type 1 diabetes (T1D) [11,12], and Goodpasture's syndrome [13], likely through their contributions to the shaping of relevant T cell repertoires. Therefore, the selection of specific T or B cell repertoires may increase the risks for developing autoimmune disease. However, other gene products appear to be required in the chain of events leading to the onset of spontaneous autoimmune disease since the expression of MHC genes alone is not predictive [14].

*Abbreviations:* T1D, type 1 diabetes; EAE, experimental autoimmune encephalomyelitis; NOD, non-obese diabetic.

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In contrast to the notion of epitope-specific initiation of disease, some have postulated that a common genetic defect may be shared among individuals with autoimmune disease [15–17]. While data supporting a common gene scenario are controversial, it is possible that certain phenotypes or characteristics related to the immune response may be shared among many autoimmune diseases. The heightened and chronic nature of the autoimmune response typically present in most autoimmune diseases [18] could be a reflection of a general defect in the regulation and contraction of host immune responses [19]. NOD mice spontaneously develop insulitis and T1D, an autoimmune disease characterized by T cell-mediated destruction of the insulin-producing beta cells of the pancreas [11]. The mechanisms that are normally responsible for the induction and maintenance of self-tolerance may be defective in these mice rendering them unable to provide adequate protection from autoreactivity and autoimmune disease. This viewpoint is supported by the spontaneous appearance of cellular and humoral immune responses to islet antigens prior to the onset of diabetes [20,21]. Previously, we demonstrated that autoimmune prone NOD mice display a defect in peripheral tolerance that is independent of their unique I–$A^{g7}$ molecule, but instead involves regulatory mechanisms such as activation-induced cell death (AICD) [22]. T cells in NOD mice may be resistant to tolerogenic signals delivered during thymic education [23] and in the periphery [22]. An overall loss of regulation in self-reactive repertoires may contribute to pathogenesis in T1D and other autoimmune diseases as well. Here, we utilized the non-obese diabetic (NOD) mouse model of T1D to address the hypothesis that a general immunological defect can predispose to autoimmune disease.

In NOD congenic mice, replacement with wild-type loci reduces the severity of insulitis, the incidence of T1D, and the overall progression of beta cell destruction, suggesting that the introduced alleles amend the dysregulated autoimmunity. In NOD.B10.Idd5 congenic mice, replacement of loci 5.1 and 5.2 with B10 alleles results in a significant decrease in the incidence of T1D (<40%)[24]. The NOD.B10.Idd9 mouse has loci 9.1, 9.2, and 9.3 replacements that provide protection from T1D, dramatically reducing the incidence to <5% [25]. The precise genes that distinguish NOD from NOD.B10.Idd9 and NOD.B10.Idd5 mice are still under investigation; however, the B10-derived loci in the congenic mice include candidate molecules related to the control of T cell expansion and chemokine receptors [24,25]. We compared the susceptibility of NOD mice to experimental autoimmune encephalomyelitis (EAE) with that of NOD.B10.Idd5, and NOD.B10.Idd9 mice to determine if the B10-derived loci, which appear to correct immune dysregulation on the NOD background, could provide protection from an inflammatory autoimmune disease that targets an organ other than the pancreas. We find that certain Insulin-dependent diabetes (Idd) alleles not only failed to protect against the induction of EAE, but also surprisingly appeared to contribute to an earlier onset and a more severe form of the disease.

## Materials and methods

### *Mice*

NOD/MrkTac, NOD.B10.*Idd5*R444N13F5 (Model #1094) and NOD.B10.*Idd9*R28N12F7 (Model #1104) mice were purchased from Taconic Farms (Germantown, NY). The mice were housed under specific pathogen-free conditions in the animal care facility at the University of Toledo. The mice were age-matched and sex-matched in all experiments.

### *Induction of EAE*

Five- to 12-week old mice were immunized subcutaneously on the upper dorsal flank with 100 μg of myelin oligodendrocyte glycoprotein peptide MOG35–55 (MEVGWYRSPFSRVVH-LYRNGK) (Cell Essentials Inc., Boston MA), emulsified in CFA supplemented with 2 mg/ml Mycobacterium tuberculosis (strain H37RA; Difco, Detroit, MI). Unless otherwise noted, the mice were also injected intraperitoneally with 200 ng of pertussis toxin (List Biological Laboratories, Campbell, CA) on day 0 and day 2. The clinical scores were recorded routinely by 1–3 blinded observers following immunization. Disease severity was scored on a five-point scale: 1, flaccid tail; 2, hind limb weakness/incomplete limb paralysis; 3, hind limb paralysis; 4, complete hind limb paralysis and partial front limb paralysis; 5, moribund and/or death.

### *TCR peptide vaccination*

NOD.B10.Idd5 mice were immunized subcutaneously with 15 μg B5 peptide (amino acids 76–101 of the murine TCR Vβ8.2 chain) emulsified in IFA, or PBS in IFA. Twelve days after B5 pretreatment, EAE was induced as described above.

### *Proliferation assay*

To measure proliferative recall responses, popliteal and inguinal lymph nodes were collected 10 days after subcutaneous immunization with MOG35–55/CFA, while spleen cells were used to measure cellular immune responses after disease onset. Lymph node cells ($5 \times 10^5$/well) or splenocytes ($8 \times 10^5$/well) were cultured in 96-well microtiter plates with 200 μl of HL-1 serum-free medium (BioWhittaker; Portland, ME) supplemented with 2 mM L-glutamine. MOG35–55 was added to a final concentration of 10.0–0.1 μg/ml, while anti-CD3 (1.0 μg/ml) was added to wells as a positive control. One μCi of [$^3$H] tritiated-thymidine (International Chemical and Nuclear, Irvine, CA) was added for the last 18 h of a 96 h culture. The cells were harvested using a Micro Cell Harvester (Skatron Instruments, Sterling, VA) and the incorporation of label was measured by liquid scintillation counting in an LKB 1205 Betaplate counter. The results were collected as mean counts per minute (cpm) of triplicate wells; the standard deviation (SD) was less than 15% in all experiments. The data are expressed as stimulation index (SI); experimental cpm/media control cpm.