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Altered susceptibility to EAE in congenic NOD mice: Altered processing of the encephalitogenic MOG35—55 peptide by NOR/LtJ mice

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Abstract NOD mice (H-2^{g7}) naturally develop autoimmune diabetes, while the congenic NOR/LtJ mice (H-2^{g7}) are resistant. To determine if defective immune regulation renders NOD susceptible to autoimmune disease, we compared MOG35—55-induced EAE in NOD mice to that of NOR/LtJ. In two of three immunization protocols, the NOR/LtJ mice developed significantly reduced indices and severity of clinical disease, in spite of an exaggerated autoimmune response to MOG35—55. Characterization of the responding T cell repertoires revealed that V beta 8+ Th cells directed toward the MOG42—55 core epitope were dominant in both strains. Interestingly, CD8+ CTL were absent or significantly reduced in MOG35—55 lymphoblasts from NOR/LtJ mice, which poorly processed the MOG39—47 CTL epitope from MOG35—55. Thus, while particular MHC class II alleles may be associated with increased risk, molecules involved in the processing of key epitopes may be influential in the progression of autoimmune disease.

Introduction

NOD mice naturally develop type 1 diabetes (T1D) [1] and are used extensively as a model of human T1D. This strain is also susceptible to inducible autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE) [2], experimental autoimmune thyroiditis [3] and systemic lupus erythematosus (SLE)-like diseases [4,5]. Given the susceptibility of the NOD mouse to autoimmune disease and their suggested propensity for developing autoimmune responses, it is possible that a broad defect in immunoregulation renders this strain susceptible to autoimmunity and overt autoimmune disease. Likewise, individuals with autoimmune disease may be genetically prone to autoimmunity [6—8]. Given that most autoimmune diseases are limited in the breadth of self antigens involved and even more so in the number of organs that become targeted, it is possible that susceptibility to certain inflammatory diseases may also be significantly

Abbreviations: T1D, type 1 diabetes; EAE, experimental autoimmune encephalomyelitis; NOD, non-obese diabetic; NOR/LtJ, non-obese resistant.

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influenced by gene expression in the target organ itself [9]. The gene products would likely be decisive in shaping the lymphocytic repertoire via direct or indirect contributions to antigenic epitope creation and/or presentation.

To test the hypothesis that functional immune regulation is disrupted in NOD mice, thus causing them to become prone to pathogenic autoimmunity, we have been investigating the mechanisms that help shape the T cell repertoire in spontaneous and inducible T-cell-mediated autoimmune diseases in the NOD mouse model. Of particular interest has been the distinct NOD MHC class II molecule, I-A^{g7}, which has been reported to have several unique properties that may contribute to increased susceptibility to autoimmunity [10—14]. In a previous study, we compared the susceptibility of NOD mice to MOG-induced EAE to that of two I-A^{g7}-expressing NOD congenic mouse strains, NOD.B10.Idd5 [15] and NOD.B10.Idd9 [16], which develop T1D at significantly reduced rates [17]. NOD mice are susceptible to a relapsing remitting form of EAE induced with myelin oligodendrocyte glycoprotein (MOG) [2]. EAE, an animal model for multiple sclerosis, is thought to be a CD4+ mediated inflammatory disease in which the myelin sheath is destroyed, resulting in the development of an ascending paralysis [18]. In contrast to T1D, we found that the NOD.B10.Idd5 and NOD.B10.Idd5 mice were not protected from EAE, but rather showed an increased severity of clinical disease to the CNS, without enhancing autoimmune pathogenesis in the pancreatic islets [17]. These findings suggested that B10-derived alleles at loci Idd5 and Idd9 in the NOD congenic mice afforded protection in a disease-specific manner, rather than by correcting a generalized defect in the regulation of the immune system. Most importantly, our data suggested that the Th response to the encephalitogenic peptide was similar in the NOD and NOD congenic mice [17] and that the I-A^{g7}-restricted response was not the determining factor in the severity of EAE.

The NOR/LtJ mouse is another strain that has been useful in the study of immune dysregulation in the NOD mouse model. NOR/LtJ mice are recombinant congenics that share the majority of their genome with NOD mice [19], with limited regions replaced with alleles from the C57BLS/J mouse [19]. While NOR/LtJ mice express the diabetogenic I-A^{g7} molecule [20], they are completely resistant to spontaneous T1D and cyclophosphamide-induced T1D [19], prompting suggestions that they are able to delete or regulate the activities of pathogenic autoimmune T cell clones. Similarly, siblings and first-degree relatives of individuals with autoimmune disease can remain healthy despite their expression of MHC alleles that have a clear association with increased risk of a particular autoimmune disease [20,21]. Here, we sought to evaluate the sensitivity of NOR/LtJ mice to MOG-induced EAE to determine if they are able to recruit and expand encephalitogenic T cells, and to compare the I-A^{g7}-restricted Th cell response to MOG in NOR/LtJ and NOD mice.

Materials and methods

Mice

NOD mice were purchased from Taconic Farms (Germantown, NY). NOR/LtJ were purchased from Jackson Laboratories (Bar Harbor, ME). The mice were housed under specific-pathogen-free conditions in the animal care facility at the University of Toledo. $(NOD \times NOR/LtJ)_{F1}$ mice were bred onsite at the University of Toledo. The mice were age-matched and sex-matched in all experiments.

Induction of EAE

EAE was induced as previously described [17]. Briefly, 5- to 12-week-old mice were immunized subcutaneously on the upper dorsal flank with 100 μg or 200 μg of myelin oligodendrocyte glycoprotein peptide MOG35—55 (MEVGWYRSPFSRVVHLYRNGK) (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 the same day as the MOG35—55 injections, and 2 days later. The clinical scores were recorded routinely by blinded observers immediately 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 or death.

Proliferation assay

Proliferative recall responses were measured as previous described [17]. Lymph node cells or splenocytes from peptide-immunized mice were cultured in 96-well microtiter plates with MOG35—55, 10.0—0.1 μg/ml, or MOG35—48 (MEVGWYRSPFSRVV), MOG38—51 (GWYRSPFSRVVHLY) or MOG42—55 (SPFSRVVHLYRNGK) (Invitrogen, Carlsbad, CA) at 10 μg/ml. 1 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 results were collected as mean counts per minute (cpm) of triplicate wells; the standard deviation (SD) among triplicates was less than 10% in all experiments. The data are expressed as stimulation index (SI); experimental cpm/media control cpm. To measure responses of T cell lines or clones, 2×10^4 T cells were cultured in the presence of 3×10^5 irradiated spleen cells and the relevant peptide antigen in 96-well plates. One hundred microliters of culture supernatant was collected 48 h later, and the cells were pulsed with [^{3}H] tritiated-thymidine.

Cytokine ELISA

To characterize the cytokine profile of MOG-reactive T cells, single-cell suspensions from draining lymph nodes or spleen cells were pooled 10 days post-immunization, or following disease onset, and cultured as described for the proliferation assay. Supernatants were removed 48 to 72 h later and tested by ELISA for the presence of IFN-γ, TNF-α, IL-4, IL-5, IL-10 and IL-13, using cytokine-specific antibody pairs (BD Biosciences, San Diego, CA and R&D Systems, Minneapolis, MN). Standards for IFN-γ, TNF-α, IL-5, IL-4, IL-10 and IL-13 (Peprotech, Rocky Hill, NJ) were added at 50 ng/ml and serially diluted. The standard deviation between duplicate wells was less than 5%.