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HLA-DQ8 is a predisposing molecule for detergent enzyme subtilisin BPN′-induced hypersensitivity

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Abstract

Several million individuals are exposed to agents in the workplace associated with atopy and asthma. Detergent enzymes have been implicated in occupationally induced hypersensitivity. However, the genetic susceptibility and T cell responses to detergent enzymes are undefined. We generated and used HLA-DQ6, -DQ8, -DR2, -DR3, and -DR4 transgenic mice to examine the immune and inflammatory components involved in the response to the detergent enzyme subtilisin BPN′. Based on in vitro and in vivo studies, for the first time, we present evidence that DQ8 is a strong susceptibility marker for BPN′-induced hypersensitivity. Only DQ8 mice showed consistent T cell responses to five immunodominant regions of BPN′ comprising peptides #14 to 16, 36–37, 42–43, 62–63, and 80–81. The DQ8 mice also developed allergic eosinophilic inflammatory reactions in the airways following intranasal instillations of this enzyme. The DQ8 mice also responded to BPN′ with a significant IgG1 and IgE production. We propose that the HLA Class II tg mice are useful for understanding allergenic responses to enzymes in humans, screening of allergenic and immunogenic properties of detergent enzymes, and for the development of modified enzymes to maintain efficient detergent qualities without allergic properties.

Keywords: Transgenic; Knockout; Allergy; Detergent; Enzyme; Eosinophils; Antigens; Peptides; Epitopes; Cytokines

Introduction

With approximately 9 million individuals exposed and potentially at risk for development of occupational asthma (OA), hypersensitivity to occupational stimuli continues to be a public health concern [1,2]. OA may be caused by high-molecular-weight sensitizers (allergens), low-molecular-weight sensitizers (chemicals), or irritant gases [3]. Washing powders in the detergent industry contain known high-molecular-weight sensitizers enzymes. These enzymes are proteins derived from bacterial or fungal organisms and are present in detergents to improve their cleansing properties. Allergies and asthma to these powdered enzymes appeared in the late 1960s in the detergent plants where bacterial proteases, subtilisins, were identified as the causative agents. Since the introduction of an advanced technology of enzyme delivery into the final product and Industry's strict control of occupational exposure to respirable enzyme dust, the incidence of immediate hypersensitivity responses and the occurrence of enzyme-associated occupational asthma among detergent workers is currently a rare event [4]. However, new occupational sensitizations in detergent plants have not been totally prevented [5,6].

HLA Class II polymorphism has been found to be associated with OA [7] including asthma and atopy involving isocyanates [8], Western red cedar [9], acid anhydrides [10], and complex platinum salt [11]. Though information has been published on Ab responses in humans, mice, and guinea pigs to detergent enzymes, the association of HLA Class II with sensitization to enzymes is unknown. BPN′ (Y217L variant

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herein referred to as BPN′), a bacterial protease from *Bacillus amyloliquefaciens*, was chosen for this study because it is a protease commonly found in liquid laundry detergents. Therefore, in order to further improve our understanding of the BPN′ allergenicity, the response to BPN′ was evaluated in transgenic (tg) mice expressing HLA-DQ6, -DQ8, -DR2, -DR3, and -DR4 genes. Several routes of Ag administration were evaluated: subcutaneous (s.c.), intranasal (i.n.), and combined intraperitoneal/intranasal (i.p./i.n.). We identified that DQ8 molecules serve as restriction elements for an immune response to BPN′. DQ8 and DQ8/hCD4 tg mice were able to recognize several epitopes on BPN′ and developed allergic inflammatory reactions in the airways following i.n. instillations of this enzyme. We show here that the BPN′ T cell epitope repertoire in DQ8 mice is not modulated by (1) two non-MHC genetic backgrounds; (2) adjuvant codelivery (but is modulated by adjuvant presence); (3) immunization route and dose of enzyme if adjuvant is used. DR4 mice responded to several epitopes using combined i.p./i.n. BPN′ application only. Preliminary data indicate that HLA Class II tg mice have similar epitopes compared to subsets of humans skin prick test positive to BPN′. This work suggests that HLA Class II tg mice can provide information on the allergenicity of enzymes and should be used as potential models to assess the safety of known and new detergent enzymes in humans.

Materials and methods

Mice

Transgenic mice expressing either HLA-DQ8 (DQA1*0301 and DQB1*0302), HLA-DQ6 (DQA1*0103 and DQB1*0601), HLA-DR2 (DRB1*1502), HLA-DR3 (DRB1*0301), or HLA-DR4 (DRB1*0401) in H-2Aβ^0 mice were generated as previously described [12–16]. The H-2Aβ^0 mice have both a spontaneous mutation in the promoter region of the H-2Eα gene (in H-2^b haplotypes) and a targeted disruption of the H-2A gene. Neither H-2Aα^b nor H-2Eβ^b molecules were detected on peripheral blood leukocytes in tg mice. The surface expression of hybrid molecules formed by H-2Aα^b, DQβ, or DRβ chains was not observed. H-2Aβ^0 mice and B10 mice served as transgene negative and background controls. DQ8 transgenic mice were mated to mCD4^0 (kindly provided by Tak W. Mak, University of Toronto) [17] and then to hCD4 tg mice (kindly provided by Richard A. Flavell, Yale University) [18] to generate the double transgenic double knockout mice [19,20]. All mice used in these experiments were between 8 and 12 weeks of age. Both males and females were equally represented in each experiment. Mice were bred and maintained in the pathogen-free Immunogenetics Mouse Colony at the Mayo Clinic (Rochester, MN). All procedures performed on the mice were in accordance with the Mayo Institutional Animal Care and Use Committee.

Anesthetic agent

Stock avertin solution (10 g of 99% 2,2,2-tribromoethanol; Aldrich Chemical Co., Milwaukee, WI) was dissolved in 10 ml *tert*-amylalcohol (Fisher Scientific, Fairlawn, NJ). One hundred microliters of stock solution was diluted in 5 ml PBS. An intraperitoneal injection (1 ml) was used for euthanization, and 0.2–0.3 ml was administered for anesthesia.

BPN′

Subtilisin BPN′ (Y217L) is a variant of the bacterial protease derived from *B. amyloliquefaciens*. Purified BPN′ was obtained from Genencor International (San Francisco, CA). A pNA assay is used to determine the active enzyme concentration.

Initially, it was determined that active enzyme was detrimental to cells in culture. Therefore, BPN′ was inactivated by a trifluoromethyketone inhibitor for in vitro use. This subtilisin inhibitor 2-(*N*-methoxycarbonyl-phenylalanyl-glycyl-alanyl)-amino-5-methyl-1,1,1-trifluoro-2-hexanone was synthesized by Procter & Gamble [21]. The inhibitor is non-cytotoxic at the concentrations used in the proliferation assays, and control wells with inhibitor alone showed no proliferation. In addition, the inhibitor was chosen as it binds to the active site and does not alter the three-dimensional structure of BPN′.

Peptides

A set of 88 overlapping peptides offset by 3 amino acids spanning the length of BPN′ was commercially synthesized by Chiron Mimotopes (Raleigh, NC) using the Multi-Pin technique [22]. The peptides were 15 amino acid residues long and had a 12 amino acid residue overlap with adjacent peptides in the panel (Table 1). Peptides were dissolved in DMSO at final concentration of 10 mM. Levels of DMSO in culture were kept below 0.3%.

Antigen immunization protocols

Mice were immunized by three different routes of exposure outlined in Fig. 1.

Subcutaneous immunization

Mice were injected s.c. with a single dose of BPN′ (40 μg) emulsified in CFA (Difco, Detroit, MI) into the tail and footpad. Control mice were dosed s.c. with CFA. Seven days post-injection, draining lymph nodes were removed for epitope mapping studies (Fig. 1A).

Repeated intranasal immunization

For i.n. immunization of transgenic mice, BPN′ was instilled (5μl/nostril, total 10 μg/10μl/mouse) in sterile endotoxin-free PBS (Gibco BRL) on days 1, 3, 10, 17, and 24 of the study to the restrained anesthetized mice (n = 4–8) [23]. Control groups of mice were treated with an equal volume of PBS in similar fashion. On day 29, spleens were obtained for in vitro proliferation assays. BAL fluids were collected as described previously [24] on day 29 of the study (Fig. 1B).