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Whole-cell pertussis vaccine protects against *Bordetella pertussis* exacerbation of allergic asthma

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Abstract

The prevalence of asthma and allergic disease has increased in many countries and there has been speculation that immunization promotes allergic sensitization. *Bordetella pertussis* infection exacerbates allergic asthmatic responses. We investigated whether whole-cell *pertussis* vaccine (Pw) enhanced or prevented *B. pertussis* induced exacerbation of allergic asthma. Groups of mice were immunized with Pw, infected with *B. pertussis* and/or sensitized to ovalbumin. Immunological, pathological and physiological changes were measured to assess the impact of Pw immunization on immune deviation and airway function. Pw immunization modulated ovalbumin-specific serum IgE production, and reduced local and systemic IL-13 and other cytokine responses to sensitizing allergen. Histopathological examination revealed Pw immunization reduced the severity of airway pathology and decreased bronchial hyperreactivity to methacholine exposure. Pw does not enhance airway IL-13 and consequently does not enhance but protects against the exacerbation of allergic responses. We find no evidence of Pw contributing to allergic asthma, but rather provide evidence of a mechanism whereby whole-cell pertussis vaccination has a protective role.

Keywords: *Bordetella pertussis*; Vaccine; Asthma; IL-13; Allergen

1. Introduction

Asthma is a chronic disease of the respiratory tract of increasing prevalence in developed societies [1]. The current understanding of allergic asthma is that it results from a breakdown in the normal tolerance to inhaled antigens, associated with Th2 cytokine production [2,3]. The inflammatory response in asthma is tightly associated with airway hyperresponsiveness, increased mucus production and an infiltration of the bronchial mucosa with CD4+ T-cells [4]. There is evidence of an altered local T-cell response in favour of Th2 cytokine release (IL-4, IL-5 and IL-13) resulting in B-cell isotype switching to IgE, recruitment of eosinophils, basophils and mast cells and production of inflammatory mediators [5]. The murine OVA model of airway hyperresponsiveness exhibits many of the features of human asthma, including airway hyperreactivity, inflammation and increased serum IgE levels [6,7]. Th2 cells secreting IL-4, IL-5, and IL-13 play a central role in initiating and sustaining the asthmatic response in this model [8]. While Th2 cells promote airway inflammation in asthma, it has been proposed that Th1 cells protect against allergic disease by antagonizing Th2 activity. Infectious diseases that induce Th1 type responses, might hamper the development of allergen-specific Th2 cells and prevent allergy [9].

Epidemiological and clinical studies have suggested a link between the relative absence of infectious diseases and the increase in allergic disorders [10,11]; this is referred to as the 'hygiene hypothesis'. It predicts that infections prevent the induction of allergen-specific Th2 cells through antagonism or the induction of regulatory T-cells, particularly during neona-

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tal and early childhood development [10,12]. However, there are data that confound this interpretation, increased IFN-γ is seen in asthmatic patients compared with normal subjects [3,13]. Allergen-specific Th1 cells also fail to counteract airway hyperresponsiveness in murine models [14]. Furthermore, several studies have suggested that viral/bacterial infections do not protect but exacerbate disease. Respiratory syncytial virus, commonly associated with lower lung infections in infancy, is known to exacerbate asthma [15,16], as does *Bordetella pertussis* [17]. Consequently, competing interpretations for the pathogenesis of asthma have been proposed [18,19].

B. pertussis is a Gram-negative bacterium and the causative agent of pertussis or "whooping cough", a respiratory disease that remains a significant cause of morbidity and mortality in infants worldwide. It is a highly contagious disease, and can occur at any age, though severe illness is more common in young un-immunized children. *B. pertussis* infection induces Th1 responses [20,21] and can be modelled by respiratory challenge of mice, which correlates well to responses in humans [22].

There has been speculation about the possible promotion of allergy by common childhood vaccinations [23,24]. A substantial proportion of children predisposed to allergy and asthma may not be fully immunized because of public apprehension surrounding immunization [25]. A number of studies have analysed the prevalence of allergic sensitization and atopic disease in relation to immunization [24,26]. Gruber et al. found that children with higher immunization coverage seemed to acquire transient protection against development of atopy in the first years of life [26]. In contrast, Hurwitz and Morgenstern suggested that diphtheria/pertussis/tetanus (DTP) immunization appeared to be associated with an increased risk of subsequent asthma or other allergies [24].

Two different types of pertussis vaccine have been employed in infant immunization programmes. The whole-cell pertussis vaccine (Pw) consists of heat/formalin inactivated virulent whole bacteria whereas the pertussis acellular vaccine (Pa) is composed of purified components of the bacteria (Pa), typically including inactivated pertussis toxin. Pw immunization has a high efficacy and is associated with the induction of antigen-specific Th1 cells [21,27,28], but has been associated with reactogenicity. In contrast Pa immunization induces a mixed Th1/Th2 response in children and in murine models, but has reduced reactogenicity [29]. It has been suggested that promotion of allergy may occur directly, by administering potentially pro-allergic vaccines, or indirectly, by hindering the Th1-promoting effect of infectious agents. Pertussis vaccination acts as an adjuvant for antigen-specific responses in laboratory animals [30]; active pertussis toxin, is known to enhance immunoglobulin E (IgE) formation in animal models [31] and has been linked with a shift toward Th2-like cytokines in humans [32,33].

Infection with *B. pertussis* modulates allergen priming and the severity of airway pathology in a murine model of allergic asthma [17] and we have previously shown that Pw immunization induces a similar immune response to infection [34] and that although variables such as route, dose and timing influence T-cell responses in animal models, Pw is a consistent inducer of Th1 responses [20]. In order to test whether immunization with Pw exacerbated asthma, we employed a well-characterized murine model of whole-cell pertussis vaccination and *B. pertussis* infection in combination with the murine OVA model of airway hyperresponsiveness. We show that although Pw induces a Th1 type immune response to *B. pertussis* infection, it does not exacerbate pathology in a model of allergic asthma. Our findings demonstrate that Pw immunization prevents *B. pertussis* enhancement of OVA-induced IL-10 and IL-13, which results in a subsequent decrease in airway hyperresponsiveness and pathology. This study finds no evidence of a mechanism to support speculation linking Pw immunization and asthma.

2. Materials and methods

2.1. Animals and experimental approach

6- to 8-week-old female BALB/c (Harlan, UK) mice were used under the guidelines of the Irish Department of Health and the research ethics committee of the National University of Ireland Maynooth. The experimental approach is outlined in Table 1, briefly groups of mice were immunized with whole-cell pertussis vaccine (Pw), infected with *B. pertussis*, and then sensitised to ovalbumin (OVA) at the peak of infection as detailed below. Control mice received similar treatment in which 0.9% (w/v) (aq) NaCl (hereafter termed Saline) replaced experimental treatment.

2.2. Immunization, sensitization and airway delivery of OVA

Four groups of at least thirty-five 6–8-week-old female BALB/c mice (Pw, PwBp, PwOVA and PwBpOVA) were immunized i.p. with 0.16 I.U. of whole-cell pertussis vaccine (Pw) (Third International Standard, 1998, pertussis whole-cell vaccine, NIBSC, UK), equivalent to 1/25th of the human dose according to the schedule outlined in Table 1. At 0 day mice were infected with *B. pertussis*, selected groups were then sensitized with ovalbumin (OVA). Sensitization involved 100 μg OVA (Grade V; Sigma, Dorset, UK) emulsified in Alhydrogel® adjuvant (Superfos Biosector, Sweden) (1 mg/mouse aluminium hydroxide) administered as 0.2 ml i.p. at 10 and 24 days. Control group (Ctrl) received saline alone (i.p.). On 35, 36, and 37 days, PwOVA and PwBpOVA sensitized mice received 10 μl containing 50 μg OVA intra-nasally (i.n.) whereas remaining groups received saline only (Table 1). All experiments were repeated at least twice.