Available online at www.sciencedirect.com
SCIENCE DIRECT®
Vaccine 23 (2005) 3181–3186
Vaccine
www.elsevier.com/locate/vaccine

Peptide induces $CD4^+CD25^+$ and IL-10^+ T cells and protection in airway allergy models

Cindy L. Zuleger, Xiaoyan Gao, Melissa S. Burger, Qili Chu, Lendon G. Payne, Dexiang Chen*

PowderJect Vaccines Inc., Middleton, WI 53562, USA

Received 27 August 2004; received in revised form 13 December 2004; accepted 14 December 2004
Available online 12 January 2005

Abstract

The purpose of this study was to evaluate whether a single peptide containing a major T cell epitope might induce peripheral tolerance in a complex allergen model. C57BL/6 mice were sensitized by intraperitoneal injection of house dust mite extract (HDM), and exposed to antigen via trachea instillation. Der p 1 peptide was administered by i.v. before or after sensitization. Lung lavage fluids were analyzed for cellular infiltration. Respiratory exposure of sensitized mice to antigen results in airway inflammation and eosinophilia. Intravenous administration of a single peptide protected sensitized mice from these changes. Further, the emergence of antigen-specific $CD25^+CD4^+$ and IL-10 secreting cell populations in DO11.10 mice was demonstrated after peptide administration. Thus, intravenous delivery of a single peptide epitope is capable of inducing peripheral tolerance and protection in a complex allergy model, possibly through regulatory T cells and bystander suppression.

Keywords: Bystander suppression; House dust mite allergy; Peptide vaccine; Peripheral tolerance; Regulatory T cells

1. Introduction

Allergic asthma is marked by airway hyperresponsiveness (AHR) and inflammation, excess mucus secretion and elevated serum IgE levels. $CD4^+$ Th2 lymphocytes in the airways of allergic individuals and cytokines (IL-4, IL-5, IL-13, etc.) secreted by these cells are thought to be important in the establishment and progression of allergic asthma [1]. IL-4 enhances the immunoglobulin class switch to IgE, regulates the expansion of IgE-dedicated B cells and the production of mast cells. IL-13 is linked to excessive mucus secretion in the airway. IL-5 promotes the differentiation, maturation and activation of eosinophils, as well as functioning as an eosinophil chemotractant. The accumulation of mast cells and eosinophils in the airways following chronic allergen exposure results in the pathology associated with the disease.

Specific immunotherapy (SIT) for allergic disease involves the subcutaneous injection of increasing doses of whole allergen extracts. The use of SIT has been limited due to the risk of anaphylactic reactions triggered by vaccine-induced cross-linking of IgE antibodies anchored on the surface of mast cells. Safer and more effective alternative therapeutic approaches are needed to combat the ever-increasing rate of allergic disorders. One of these approaches involves induction of peripheral tolerance by systemic or mucosal delivery of short synthetic MHC class II binding peptides that contain T helper cell epitopes [2–6]. Peptide-based allergy vaccines may be safer because they do not contain IgE-binding epitopes. However, designing an effective peptide vaccine for human immunization is complicated by the polymorphism of the human MHC molecules and the antigenic complexity of allergens. Often atopic individuals are allergic to multiple antigen sources and each allergen source may have multiple components. There are many types of MHC molecules and each recognizes peptides of different amino acid sequence. For this reason, recent studies have used overlapping peptides spanning large portions of the allergen molecule to immunize

* Corresponding author. Present address: PATH, 1455 NW Leary Way, Seattle, WA 98107, USA. Tel.: +1 206 788 2340; fax: +1 206 285 6619.
E-mail address: dchen@path.org (D. Chen).

0264-410X/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2004.12.012

humans [6–8]. It is not practical for a peptide-based allergy vaccine to encompass all T cell epitopes matching each MHC type. The aim of this study was to determine if immunization with a single peptide is sufficient: (1) to induce tolerance and protection against multi-component allergens in a mouse strain of appropriate MHC background and (2) to determine the immune mechanism of protection. This information has important implications for the rational design of peptide vaccines.

2. Materials and methods

2.1. Allergens and formulations

Whole body *Dermatophagoides pteronyssinus* extract (house dust mite (HDM)) was purchased from Greer Laboratories (Lenoir, NC). Endotoxin-free ovalbumin (OVA) (ICN, Costa Mesa, CA) was used as a model allergen. Der p 1 peptide 114–128 (p114–128) (SNYCQIYPPNANKIR) and OVA peptide 323–339 (p323–339) (ISQAVHAAHAEINEAGR) were synthesized by Biosource International (Hopkinton, MA) with a free carboxyl terminus and HPLC purified. Aluminum hydroxide (alum) was purchased from Accurate Chemical (Westbury, NY).

2.2. HDM allergy models

Six- to eight-week-old female C57BL/6 mice (Charles River, Wilmington, MA) were used. The murine allergy model involved a sensitization and a challenge step. For sensitization, each mouse was injected intraperitoneally (i.p.) with 10–50 μg HDM adsorbed to 1–4 mg alum in 0.5 ml volume using a 26-gauge needle once or twice at weekly intervals. Mice were challenged by spraying intratracheally (i.t.) a saline solution containing 10 μg of HDM into the lungs. Briefly, mice were anesthetized by i.p. injection of ketamine (1.5 mg) and xylazine (0.36 mg). A blunt-end lavage needle attached to a pump-operated Hamilton syringe via a 12-cm long Tygon tubing (80 mm inside diameter) was used for spraying the challenge solution. By pressing the pump, 10 μl solution was dispensed into the lungs as a fine aerosol mist via the lavage needle placed at the opening of the trachea. Each mouse received five sprays, for a total of 50 μl solution containing 10 μg of HDM total protein, to ensure uniform distribution of the challenge solution throughout the lungs. Unless otherwise noted, the challenge was repeated 48 h later and the mice were euthanized with CO_2 after an additional 24 h. Bronchoalveolar lavage fluids (BALF) were collected for differential cell counts and cytokine analysis, and the lungs were collected for histological examination. Blood was collected prior to challenge via retro-orbital bleeding. Unless otherwise noted, eight mice per group were used.

For tolerance induction, mice were injected i.v. via the tail vein with 5 μg p114–128 or p323–339 dissolved in 0.2 ml sterile saline using a 30-gauge needle. In the prophylactic model, the injections were given three times at weekly intervals and were completed 1 week before sensitization. In the therapeutic model, mice received one, two or three injections at varying times after sensitization.

Mice were cared for and maintained under the "Guide for Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press (revised 1996).

2.3. OVA transgenic model

DO11.10 TCR transgenic mice, mated on the Balb/c background, were purchased from Jackson Laboratory (Bar Harbor, ME) and used to study the T cell responses to peptide immunization. The DO11.10 TCR line is transgenic for a receptor that recognizes the OVA peptide 323–339 in the context of I-A^d MHC molecules. The monoclonal antibody, KJ1-26, is specific for the DO11.10 transgenic TCR. Mice were either injected i.v. with 5 μg p323–339 on days 0, 7 and 14, or sensitized by i.p. injection of 10 μg OVA adsorbed to 0.5 mg alum on days 0 and 14.

2.4. Intracellular cytokine and T cell marker staining

Spleens of transgenic mice were harvested on Day 21 and 1×10^6 splenocytes per well of 96-well flat-bottom plates (Falcon BD, Franklin Lakes, NJ) were stimulated with 10 μg/ml OVA in complete RPMI-1640 containing 10% fetal bovine serum (FBS) (Harlan Bioproducts, Indianapolis, IN) and 1:16,000 dilution GolgiStop (BD Biosciences, San Diego, CA) at 37 °C for 24 h. Control wells contained medium alone. Cells were then collected, transferred to 96-well round-bottom plates (Falcon BD) and incubated on ice for 30 min with Cy-Chrome-labeled anti-CD4, allophycocyanin-labeled anti-CD25 and fluorescein isothiocyanate-labeled anti-KJ1-26 antibodies (BD Biosciences) in 100 μl FACS buffer (PBS containing 2% bovine serum albumin and 0.02% sodium azide). The cells were washed three times in FACS buffer, and permeabilized with 100 μl Cytofix/Cytoperm (BD Biosciences) on ice for 30 min. Following three washes with Perm Wash (BD Biosciences), cells were stained with phycoerythrin-labeled anti-IL-10 antibody in 100 μl of Perm Wash on ice for 30 min. Cells were washed two times with Perm Wash and once with FACS buffer. Splenocytes from the same transgenic mice were also stained without stimulation for surface markers in FACS buffer followed by fixation with 2% paraformaldehyde. $CD4^+$ KJ1-26^+ cells were analyzed on a FACScaliber (BD Biosciences) flow cytometer using CellQuest software (BD Biosciences).

2.5. In vitro T cell proliferation

Single-cell suspensions were prepared from the spleens of C57BL/6 or DO11.10 mice and cultured in RPMI-1640 with