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No evidence of cross-reactivity of human antibodies to a 33-mer peptide of the alpha-gliadin component of gluten with *Bordetella pertussis* pertactin

Qiushui He[a,*], Matti K. Viljanen[b], Ari E. Hinkkanen[c], Heikki Arvilommi[a], Jussi Mertsola[d], Markku Viander[b]

[a] *Department of Human Microbial Ecology and Inflammation, National Public Health Institute, Kiinamyllynkatu 13, 20520 Turku, Finland*
[b] *Department of Medical Microbiology, University of Turku, Turku, Finland*
[c] *Department of Biochemistry and Pharmacy, Abo Akademi University, Turku, Finland*
[d] *Department of Pediatrics, University of Turku, Turku, Finland*

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Abstract

A 33-mer peptide of the α-gliadin component of gluten was recently identified as primary initiator of the inflammatory response to gluten in coeliac disease (CD) patients. This proline–glutamine-rich peptide (PG-peptide) is highly homologous to internal sequence of pertactin, an immunogenic protein of *Bordetella pertussis*. Using enzyme immunoassays, we measured serum antibodies to pertactin and to PG-peptide in 167 Finnish subjects including pertussis vaccine recipients and pertussis patients, CD and non-CD patients and healthy individuals. We found no cross-reactivity between human antibodies to the two different components, suggesting that neither pertussis immunization nor disease contributes to the pathogenesis of CD.

Keywords: *Bordetella pertussis*; Vaccination; Pertactin; Antibody; Coeliac disease; Gliadin; Peptide

1. Introduction

Coeliac disease (CD) is a gluten-induced autoimmune enteropathy, which occurs in genetically susceptible individuals. The disease is one of the most common lifelong disorders in Europe and the United States [1]. A recent study showed that the prevalence of CD is 1 case in 99 among Finnish schoolchildren [2].

A 33-mer peptide, LQLQPFPQPQLPYPQPQLPYPQPQ-LPYPQPQPF, of the α-gliadin component of gluten was recently identified as primary initiator of the inflammatory response to gluten in CD patients [3]. This proline–glutamine-rich peptide (PG-peptide) is produced by normal gastrointestinal digestion of wheat gluten proteins. The PG-peptide is extremely resistant to proteolytic degradation, contains six partly overlapping copies of three different DQ2-restricted T cell epitopes, and is superstimulatory for T cells [3]. It reacts with tissue transglutaminase (tTG), a ubiquitous cytoplasmic enzyme and the major autoantigen in CD, and is presented together with tTG to the immune system resulting in antibody formation and inflammatory T cell response characteristic for CD [4]. The PG-peptide is highly homologous to the internal sequence of pertactin (Prn), an immunogenic protein of *Bordetella pertussis*.

B. pertussis, a small gram-negative coccobacillus, is the causative agent of pertussis or whooping cough. Pertussis is one of the 10 most common causes of death from infectious disease worldwide [5,6]. Immunization is the most effective method for the prevention and control of pertussis and has been used successfully for decades [5,6]. To date, both whole-cell and acellular pertussis vaccines are available. The acellular vaccines contain purified antigens of *B. pertussis*. Prn is a 69 kDa outer membrane protein of *B. pertussis* and

* Corresponding author. Tel.: +358 2 331 6634; fax: +358 2 331 6699.
E-mail address: qiushui.he@ktl.fi (Q. He).

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contributes to protective immunity in both animals and humans [7–10]. Prn is included in many of the acellular vaccines. Prn contains two immunodominant regions, composed of repeating units of five (GGXXP) or three (PQP) amino acids, namely region 1 and region 2, respectively [11]. Sequencing analysis of Prn gene of many *B. pertussis* strains isolated in different countries showed that the region 2 of Prn is very conserved and no variation was found in the region. The homology observed between PG-peptide and Prn occurs in the region 2 [3].

The development of autoimmune diseases following immunization has been a matter of debate [12]. The sequence homology between Prn and PG-peptide associated with CD motivated us to investigate the cross-reactivity between human antibodies to PG-peptide and to Prn induced after pertussis immunization or disease.

2. Materials and methods

2.1. Subjects and samples

We recruited 167 Finnish subjects (60 pertussis vaccine recipients, 32 culture-confirmed pertussis patients, 19 serologically defined CD patients, 16 serologically defined non-CD patients and 40 healthy controls). There were two groups of vaccine recipients: (1) 30 randomly selected from 150 healthy infants who had received an immunization course of three primary doses of DTaP vaccine that contains 25 μg of pertussis toxin, 25 μg of filamentous hemagglutinin and 8 μg of Prn (Infanrix™ GlaxoSmithKline Biologicals, GSK) at the ages of 3, 4 and 5 months [13]; and (2) 30 randomly selected from 104 healthy children who had received a booster dose of Infanrix™ at 2 years of age [13]. All the children had also received three primary doses of Infanrix™ at the ages of 3, 4 and 5 months. The blood samples were taken before and 1 month after the primary or booster immunization. For the 32 culture-confirmed pertussis patients (male:female ratio, 15:17; age range, 5–61 years; median, 11 years), two blood samples were obtained and the median interval between the two blood samples was 26.5 days (range, 12–84 days). Nineteen tTG IgA and anti-gliadin IgA antibody positive sera from patients suspected with CD (male:female ratio, 8:11; age range, 1–73 years; median, 49 years) were selected from consecutive serum samples sent to the Department of Medical Microbiology, University of Turku for tTG antibody measurement in 2002. According to our criteria, these patients have a serologically defined CD, and are referred here as CD patients. Serum IgA deficiency was not found in the CD patients. Sixteen non-CD patients (male:female ratio, 6:10; age range 8–75 years, median, 55.5 years) were selected from those who were suspected to have CD but not serologically confirmed at the Department in 2002. Forty healthy controls (male:female ratio, 10:30; age range, 23–57 years; median, 35.5 years) were recruited from the personnel of the National Public Health Institute, Turku.

2.2. Determination of serum antibody

The measurements of serum IgA or IgG antibodies to Prn were performed by enzyme immunoassays (EIA), as described elsewhere [14]. The coating antigens were purified Prn (5 μg/ml), kindly provided by GSK. The sequence of PG-peptide was LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF, representing the residues 56–88 of the α-gliadin component of gluten [3]. The linear PG-peptide was labelled with biotin at its N-terminal and was synthesized by Medprobe, Oslo, Norway. Streptavidin coated plates (DELFIA® plates, Perkin-Elmer, Turku, Finland) were used for the PG-peptide EIA. For EIA using PG-peptide as coating antigen, the reaction conditions, including the concentration of PG-peptides ranging from 10 to 50 ng/ml were first optimised. Five sera were used for the optimisation, and they included two from serologically defined CD patients, two from non-CD patients and the standard serum pool used for our in house pertussis EIA at the National Public Health Institute, Turku. The standard serum pool was composed of serum samples highly positive for pertussis. The final coating concentration of PG-peptide was with 0.1 μg/ml.

Test serum samples were diluted to 1:100. Anti-Prn IgG antibodies of vaccine recipients were measured at the laboratory of GSK. The titres were expressed as ELISA unit/ml and assay cut-off was 5 ELISA unit/ml. In statistical analyses values below 5 ELISA unit/ml were regarded as 2.5 ELISA unit/ml. For the EIA used at National Public Health Institute, Turku, anti-Prn or anti-PG-peptide antibodies were expressed as relative EIA units (1 EIA unit, 1:100 of the corresponding antibody concentration in the standard serum pool). A negative serum pool was also included. All serum samples were stored at −70 °C until tested and were tested in duplicate.

2.3. Statistical analysis

Comparisons were made with Student's *t*-test or with rank correlation coefficient. *P* values < 0.05 (two-sided) were considered significant.

3. Results

3.1. IgG antibodies to pertactin

A significant increase was found in the level of anti-Prn IgG antibodies in vaccine recipients before and 1 month after the primary immunization (Fig. 1A, mean 10.2 ELISA unit/ml [S.D. 16.3] versus 192.6 [169.2], $P < 0.001$) or before and 1 month after booster immunization (Fig. 1B, 24.2 [25.1] versus 1776.9 [976.9], $P < 0.001$). For patients with culture-confirmed pertussis, two blood samples were obtained and the median interval between the two blood samples was 26.5 days. A significant increase was found in the level of anti-Prn IgG antibodies in these patients (Fig. 1C, 20.6 ELISA unit [28.9] versus 61.5 [22.5], $P < 0.001$). The mean of anti-Prn