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Pertussis specific cell-mediated immune responses ten years after acellular pertussis booster vaccination in young adults

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ABSTRACT

Background: One of the reasons for pertussis resurgence is waning immunity. Both humoral and cell mediated immunity (CMI) are essential for protection. The aim of this study was to evaluate CMI responses after acellular pertussis vaccination in young adults.
Methods: Fifty-seven young adults were followed for ten years after a diphtheria-tetanus acellular pertussis (dTpa) booster vaccination. A second booster was administrated at year 10. CMI was determined from peripheral blood mononuclear cells (PBMC) stimulated with vaccine antigens pertussis toxin (PT), filamentous hemagglutinin (FHA) and pertactin (PRN) before and one month after the second vaccination, using proliferation and IFN-γ and IL-17 ELISpot. In addition, the response to ten selected cytokines was measured from 14 subjects.
Results: Before the booster dose, positive proliferation was recognized in 51%, 53% and 89% of the subjects against PT, PRN and FHA, respectively. One month after, the positivity rate increased to 81%, 81% and 96%. Although the number of IFN-γ and IL-17 secreting cells was increased, the expression of most of the tested cytokines was found to be downregulated. After PT stimulation, only one (7.1%) subject had increased production in all cytokines, whereas six (42.9%) had decreased production of all cytokines. Ten subjects (71.4%) had decreased concentration of IFN-γ, the cytokine important for pertussis protection.
Conclusions: CMI persists even when antibodies have decayed, and acellular pertussis vaccine enhances the CMI response. Further studies are needed to illustrate what factors cause the low production of some important cytokines.

1. Introduction

Pertussis has been re-emerging in several industrial countries during the past years. Countries such as the USA and the United Kingdom have witnessed the resurgence of pertussis with large epidemics [1,2]. There are several reasons for *Bordetella pertussis*, the causative agent of pertussis, to continue circulating. One reason is the fact that immunity against acellular vaccine wanes over time. Most of these industrial countries have used acellular pertussis (aP) vaccines since the 1990s, when aP replaced the traditional whole cell vaccines (wP) due to its higher reactogenicity. However, this change has not been able to control *B. pertussis* circulation. Although the mechanism behind the protection against pertussis has not been fully understood, it is believed that both humoral and cell mediated immune (CMI) responses are needed for the protection [1,3]. Clinical trials in the 1990s indicated that antibodies against acellular pertussis vaccine antigens such as pertussis toxin (PT), fimbriae (fim) and filamentous hemagglutinin (FHA) correlate with protection [4,5]. The acellular vaccine is known to produce high antibody titers against vaccine antigens, but the amount of antibodies decreases sharply already within a few years after the vaccination [6]. However, immunized children with low levels of antibodies might be protected [7,8]. The cell-mediated immunity (CMI), mediated by T and B cells, has been shown to contribute to

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protection [7,9]. The aP and wP vaccines differ in their T helper (Th) cell responses. Acellular vaccines are known to drive the immune responses to mixed Th2/Th1 direction whereas the wP vaccine induces Th1 type of immune reaction, which resembles more that of a natural infection [3]. Cytokine network is an essential part of the innate and adaptive immune responses. They have a notable role in immune responses after pertussis infection, as well as vaccine responses. The induction of interferon (IFN)-γ production by Th1 cells is important for the recovery from *B. pertussis* infection [10]. Similarly to infection, the whole cells vaccine induces high IFN-γ secretion, whereas the acellular vaccine does so only faintly [3]. More recently, Th17 cells have been found to mediate the protection after acellular vaccination in mice. Additionally Th1 and Th17 immunity seems to be more efficient in protection against pertussis [11].

Due to the rapid waning of humoral immune response after acellular vaccination, it is important to search for other possible surrogates of protection. The aim of this study was to investigate CMI responses before and one month after a second booster vaccine in young adults, who had received a diphtheria, tetanus, acellular pertussis (dTpa) booster vaccine ten years previously. Cytokine determination was performed to investigate several types of cytokines; the Th1-type (IFN-γ, IL-2, TNF-α, IL-12), Th2-type (IL-4), Th17-type (IL-17), pro-inflammatory (IL-8 and IL1B), regulatory IL-10 and inflammatory IL-6.

2. Materials and methods

2.1. Cohort

The initial study started in 1997, Turku, Finland. Five hundred and ten adolescents, aged 11–13 years, were recruited to receive a single booster dose of acellular pertussis vaccine (Boostrix, GlaxoSmithKline). The cohort was followed-up at 3, 5 and 10 years after the booster vaccination. At the 10-year follow-up, the same cohort was invited to receive an additional dTpa booster vaccine and 82 subjects enrolled in the study [12]. From the ten-year follow-up, whole blood samples were collected before the booster vaccination and one month after. A total of 57 subjects were included. All 57 were included in the proliferation assay, 21 in the IFN-γ ELISpot assay and 14 for the IL-17 ELISpot assay. Due to the limited number of cells, IL-17 was only tested in cells collected one month after the vaccination. Fourteen subjects were included for the cytokine measurement.

All subjects received four doses of DTwP vaccine in their childhood.

2.2. Cell proliferation assay

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized whole blood by density gradient centrifugations using Ficoll-Paque. Fresh cells were cultured immediately in supplemented RPMI medium, as described before [13]. Shortly, RPMI included 10% heat-inactivated human AB-serum and 1% glutamine. To avoid any contamination, the medium was supplemented with penicillin (10,000 U/ml), streptomycin (10 mg/ml) and gentamycin (50 μg/ml). 1×10^5 cells/well were cultured with the purified antigens: heat-inactivated pertussis toxin (hPT) 1 μg/ml, pertactin (PRN) 2.5 μg/ml, filamentous hemagglutinin (FHA) 1 μg/ml. Heated PT was used to avoid its mitogenic activity. Pokeweed mitogen (PWM) 1:400 dilution was used as a positive control. Spontaneous response was measured from unstimulated PBMCs (medium, M).

PBMCs were incubated for 6 days in +37 °C and 5% CO_2. Sixteen hours before the end of incubation, ^{3}H-thymidine (0.5 μCi) was added. The radioactivity was measured using a Beta counter.

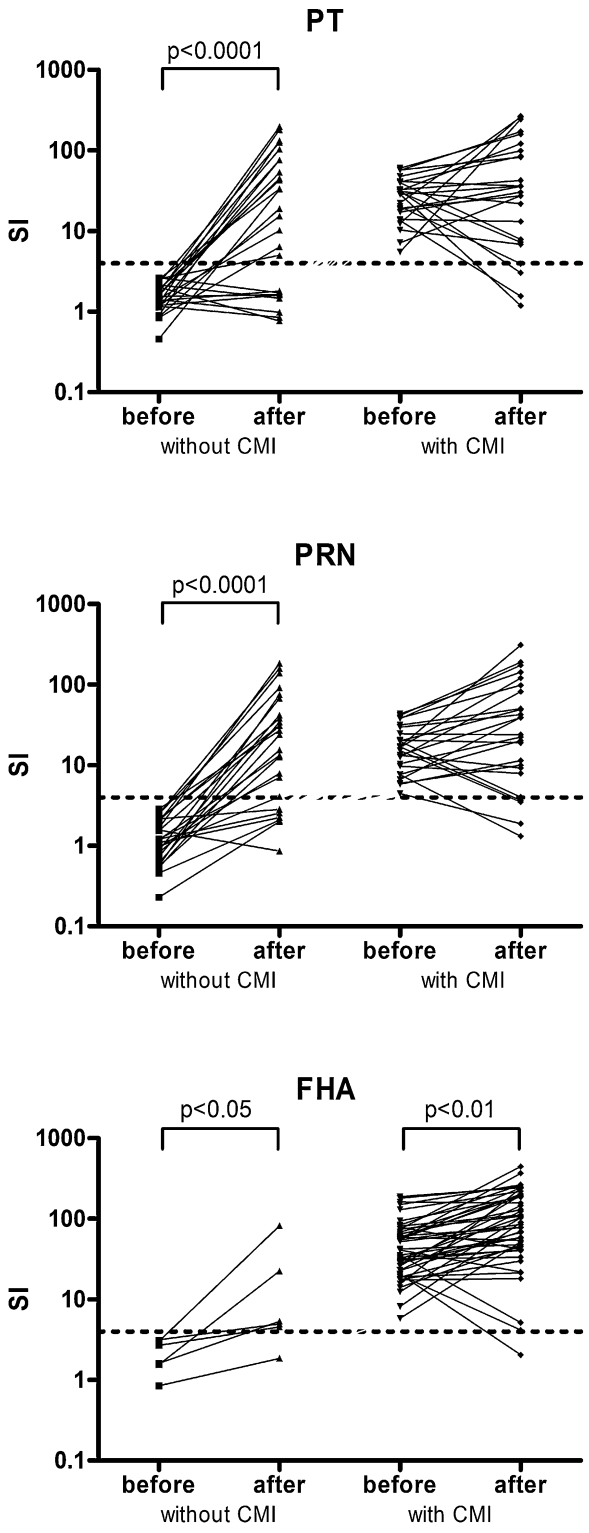

Fig. 1. Individual responses before and one month after the acellular booster vaccination against pertussis toxin (PT), pertactin (PRN) and filamentous hemagglutinin (FHA). Subjects were divided into two groups; subjects without positive cell mediated immune (CMI) response (SI < 4) and with positive CMI (SI ≥ 4) before the acellular pertussis booster vaccination. Dash line indicates the SI = 4. *p*-Values were calculated with Mann–Whitney test, $p < 0.05$ was considered as a significant difference.