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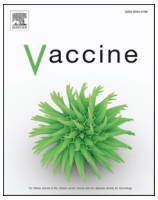

Association of baseline cytokines with antibody concentrations after diphtheria-tetanus-acellular pertussis booster vaccination in Finnish children

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ABSTRACT

Background: Despite extensive vaccinations, pertussis remains endemic and epidemic in multiple countries. The persistence of cases can be partly attributed to the significant individual variation in vaccine responses. This study evaluated the association of baseline cytokines (before booster vaccination) on antibody concentrations to Tdap-vaccine antigens.

Methods: Healthy Finnish children (7–10y, $n = 36$), adolescents (11–15y, $n = 37$), young adults (20–34y, $n = 25$), and older adults (60–70y, $n = 23$) received a Tdap3-IPV booster. Serum antibodies against pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (Prn), fimbriae 2/3, diphtheria toxoid (DT), and tetanus toxoid (TT), as well as PT neutralizing antibodies were measured before, one month, and one year after the booster. Baseline serum concentrations of IFN-γ, IL-2, IL-5, IL-10, IL-13, IL-17 A and IL-17F were determined.

Results: The proportion of detectable and undetectable baseline cytokines varied between age groups 58.3 % of children had a higher proportion of detectable IL-5, IL-10, IL-13, and IL-17F compared to adolescents (IL-5, 37.8 %; IL-10, 48.6 %; IL-13, 48.6 %; IL-17F, 37.7 %), young adults (IL-5, 36.0 %; IL-10, 28.0 %; IL-13, 36.0 %; IL-17F, 44.0 %), and older adults (IL-5, 26.1 %; IL-10, 21.7 %; IL-13, 39.1 %; IL-17F, 30.4 %). IFN-γ had a lower detectability in children (44.4 %) and young (40.0 %) and older adults (39.1 %) in contrast to adolescents (62.2 %). IL-2 was undetectable in all age groups while the proportion of detectable IL-17 A decreased with age. A mixed model showed that undetectable baseline levels of IFN-γ, IL-2, IL-10, and IL-17 A were associated with higher antibody concentrations in children before and after vaccination, particularly against PT. Positive associations were observed in adolescents for anti-TT concentrations and young adults for anti-FHA IgA concentrations.

Conclusion: These findings indicate a possible role of existing cytokines in pertussis booster antibody concentrations in children and warrant further studies in different populations. However, the results should be interpreted with caution as the number of subjects is limited.

Abbreviations: aP, acellular pertussis vaccine; BERT, Booster Pertussis Vaccine Study; DT, diphtheria toxoid; FHA, filamentous hemagglutinin; minDC, minimum detectable concentration; Prn, pertactin; PT, pertussis toxin; PTNA, PT neutralizing antibodies; r_s, spearman correlation coefficient; TT, tetanus toxoid; wP, whole-cell pertussis vaccine.

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1. Introduction

Pertussis is a highly infectious respiratory disease that poses a significant health burden. Full vaccination with either whole-cell (wP) or acellular (aP) vaccines, including the three-dose primary series and booster(s), is needed to protect against the disease. Despite the rising vaccination rates over the past decades, pertussis epidemics remain in many countries. Recent seroprevalence studies in European countries studied IgG antibodies to pertussis toxin (PT) and discovered a high circulation of the bacterial pathogen in adults [1,2]. This persistence of pertussis cases can be attributed to the significant individual variability in vaccine response, which usually varies between 10- to 100-fold between individuals depending on the type of vaccine [3]. More importantly, approximately 2–10 % of healthy individuals fail to mount sufficient antibody responses toward routine vaccination, reflecting a variability in vaccine-derived humoral and cell-mediated immune responses important for protection [4]. This variability was also observed with pertussis vaccinations, as shown in Finnish, British, and Dutch cohorts who received a diphtheria-tetanus-acellular pertussis booster vaccine (Tdap) [5–7].

Multiple studies have shown that pre-existing immunity, including baseline immune B and T cell population and enriched genes, positively and negatively affected influenza, COVID-19, hepatitis B, and pneumococcal vaccine responses [8,9]. It is known that the state of the host immune system before vaccination can be influenced by exposure to multiple internal or external factors, which include infection and vaccinations, and these effects are shown to be independent of other intrinsic factors such as age and gender. Consequently, the type and concentration of antibody, cytokine, and immune cell populations at baseline result in significant individual variability in vaccine responses [8,9]. A recent study in a Finnish cohort demonstrated that high concentrations of aP-antigen-specific antibodies led to higher antibody responses after an aP booster in pregnant women [10]. Pre-existing B-memory cells were also associated with higher post-vaccination B-memory cells specific to PT and pertactin (Prn) [7]. However, there is increasing evidence that in addition to humoral responses, cell-mediated immunity plays a vital role in pertussis vaccine effectiveness and the duration of immunity [11]. Thus, there is a clear need to identify other immunological factors that underpin the mechanisms contributing to hypo-responsiveness toward pertussis vaccination.

The analysis of baseline levels of certain cytokines may provide useful insight into the mechanisms contributing to generating a protective antibody response against pertussis antigens. We have recently reported the results from a phase IV longitudinal intervention and multicenter trial that characterized the innate and adaptive immune responses after an aP booster in different age groups in Finland, the Netherlands, and the UK [5,6]. In this study, the possible relationship of baseline cytokines on antibody concentrations post-booster was evaluated from the respective cohort of different-aged Finnish subjects.

2. Methods

2.1. Study design

The present study is a sub-analysis of the Booster Pertussis Vaccine Study (BERT), a phase IV longitudinal intervention and multicenter study conducted across the UK, the Netherlands, and Finland to analyze the innate and adaptive immune responses after an aP booster. The samples analyzed in this study are from the Finnish arm of the study, conducted by the University of Turku and Turku University Hospital. This clinical trial has been registered in the EU Clinical Trial database (EudraCT number 2016–003678-42) and was approved by the Ethics Committee of the Hospital District of Southwest Finland (ETMK Dnro: 129/1800/2017). The methodology and findings of this trial have been thoroughly described elsewhere [5]. In brief, four cohorts of different age groups were recruited (N = 124): Study cohorts comprised of 37 children and 37 adolescents aged 7–10 years and 11–15 years, respectively, and of 25 young adults and 25 older adults aged 20–34 years and 60–70 years, respectively. All participants received a Tdap3-IPV vaccine (Boostrix™-IPV by GlaxoSmithKline) at day 0 (baseline), which consists of tetanus toxoid (TT) with reduced antigen diphtheria toxoid (DT) and the aP vaccine adsorbed onto aluminum. Serum samples were collected before vaccination and on days 28 and 365 post-vaccination. Out of 124 BERT subjects, sera from 36 children, 37 adolescents, 25 young adults, and 23 older adults were measured for cytokine concentrations. The demographics of the included subjects are shown in Table 1.

2.2. Analysis of vaccine response

Serum IgG and IgA antibody concentrations were measured at days 0, 28, and 365 against vaccine antigens PT, filamentous hemagglutinin (FHA), Prn, DT, and TT by fluorescent-bead-based multiplex immunoassay as described previously [5]. All antibody concentrations were reported as international units per milliliter (IU/mL). Pertussis toxin neutralizing antibody (PTNA) titers at each time point were measured previously using the Chinese hamster ovary cell assay [6]. The reported PTNA was defined as the reciprocal of the dilution without the presence of clusters.

2.3. Quantification of cytokines

All serum samples were stored at −20 °C until cytokine measurement. The serum cytokine concentrations at baseline were measured with the Milliplex® Map Human Th17 Magnetic Bead Panel (Merck KgA, Darmstadt, Germany) that assessed Th17 (IL-17 A and IL-17F), Treg (IL-10), Th1 (IFN-γ), Th2 (IL-5 and IL-13), and IL-2 cytokines according to the manufacturer's protocol. Briefly, sera, standards, and controls were included on each plate and were incubated overnight. Cytokine levels were measured with Bio-Plex 200 (Bio-Rad Laboratories, Hercules, CA, USA) using capture beads coated with antibodies specific to the cytokine of interest. The concentration of cytokines in the test samples was determined using standard calibration dilutions, and cytokine concentrations were calculated automatically using the Bio-Plex Manager software (Bio-Rad Laboratories, Hercules, CA, USA). Cytokine concentrations that were below the minimum detectable concentration (minDC) based on kit detection limits were assigned half of the minDC value. These values were 0.90 pg/mL, 2.55 pg/mL, 0.60 pg/mL, 0.15 pg/mL, 1.20 pg/mL, 1.05 pg/mL, and 4.50 pg/mL for IFN-γ, IL-2, IL-5, IL-10, IL-13, IL-17 A and IL-17F, respectively.

2.4. Data and statistical analyses

All statistical analyses were performed using JMP® Pro, version 17.0.0 (SAS Institute Inc., Cary, NC, 1989–2023). Data were checked for normality using the Shapiro-Wilk test, where all non-normal distributed data were log-transformed. Spearman correlation (r_s) was used to assess

Table 1
Study population demographics*.

			Study groups	
	Children, (n = 36)	Adolescents, (n = 37)	Young Adults, (n = 25)	Older Adults, (*n* = 23)
Age (year), median [IQR]	9.2 [8.2–9.7]	13.8 [12.7–14.7]	30.8 [28.9–32.7]	64.20 [62.8–65.4]
Female sex, *n* (%)	18 (50)	19 (51)	21 (84)	21 (91)
Primary vaccination				
wP *n* (%)	0 (0)	18 (49)	25 (100)	23 (100)
aP *n* (%)	36 (100)	19 (51)	0 (0)	0 (0)

* In Finland, wP vaccine was replaced by aP vaccine in 2005. wP, whole cell pertussis vaccine; aP, acellular pertussis vaccine.

the relationship between baseline cytokines, age group, and antibody levels at D28 and D365. The cytokine concentrations at baseline were compared between age groups using the Kruskal-Wallis test. The proportion of cytokine detection was compared between age groups using the Chi-squared test. The effect of baseline cytokines on antibody concentrations was analyzed with a repeated-measures mixed model for each antigen-specific antibody response using an unstructured covariance structure. In this model, subjects with cytokines below and above the minDC were defined as undetectable and detectable, respectively. The use of categorical variables in the model is due to the high proportion of subjects having undetectable cytokines and the non-linear relationship between baseline cytokine concentrations and antibody concentrations. Each model consisted of fixed effects for the time, age group, baseline cytokine detection, an age group-by-baseline interaction term, and a time-by-baseline cytokine detection interaction term. The model also included random effects for subjects to account for the correlation among measures from the same subject. All pairwise comparisons with Tukey's HSD correction were used to compare the mean differences (MD) of antibody concentrations between each time point and baseline cytokine detection values. All *p*-values were corrected with Bonferroni correction. Both original and adjusted (p_{adj}) p-values were reported. All adjusted p-values of more than 1 is reported as 1. Two-sided p values of <0.05 were considered significant, and the corresponding MD and 95 % confidence intervals (CI) were reported. The results were illustrated with least means squares interaction plots. Plots illustrating the baseline cytokine detection factor include pooled antibody concentrations of all three time points to assess the effect of only the baseline cytokine detection factor on antibody concentrations. Plots with time-by-baseline cytokine detection interaction term illustrates the kinetics of antibody concentrations between subjects with detectable and undetectable baseline cytokines.

3. Results

3.1. Antibody responses

The kinetics of antibody responses after the aP booster are shown in Supplementary Fig. 1 [5,6]. Briefly, IgG antibodies to all vaccine antigens and PTNAs increased in all age groups. The antibody concentrations decreased one year post-vaccination compared to day 28. However, levels at day 365 remained significantly higher than those at baseline. Compared to IgG, the increase in IgA levels was more modest. Individual variations in vaccine responses between subjects were observed.

3.2. Baseline cytokine levels

The baseline cytokine profiles were assessed for each age group (Table 2). 58.3 % of children had detectable IL-5, IL-10, IL-13, and IL-17F concentrations. Meanwhile, the proportion of subjects with detectable cytokines was notably lower in adolescents (IL-5, 37.8 %; IL-10, 48.6 %; IL-13, 48.6 %; IL-17F, 37.7 %), young adults (IL-5, 36.0 %; IL-10, 28.0 %; IL-13, 36.0 %; IL-17F, 44.0 %), and older adults (IL-5, 26.1 %; IL-10, 21.7 %; IL-13, 39.1 %; IL-17F, 30.4 %). However, IFN-γ, IL-2, and IL-17 A did not have the same age-dependent trends observed above: children had detectable concentrations of IFN-γ, IL-2, and IL-17 A in 44.4 %, 36.1 %, and 47.2 % of cases. Respective values were 62.2 %, 27.0 %, 43.2 % for adolescents, 40.0 %, 16.0 %, 36.0 % for young adults, and 39.1 %, 17.4 %, and 17.4 % for older adults. Quantitatively, age was found to be negatively correlated with baseline IL-2 ($r_s = -0.241$, $p = 0.007$, $p_{adj} = 1$), IL-5 ($r_s = -0.212$, $p = 0.020$, $p_{adj} = 1.00$), IL-10 ($r_s = -0.231$, $p = 0.011$, $p_{adj} = 1.00$), and IL-17 A ($r_s = -0.209$, $p = 0.022$, $p_{adj} = 1.00$) concentrations. Despite this, the proportion of detectable and undetectable cytokines at baseline did not significantly vary between age groups (Table 2), except IL-10 ($p = 0.0126$, $p_{adj} = 0.27$). Additionally, median values of these cytokine concentrations in subjects were not statistically different between age groups (Table 3) except for IL-2 ($p = 0.044$, $p_{adj} = 0.92$). Nevertheless, multiple comparisons of IL-2 cytokine concentrations between age groups did not reveal significant differences.

The relationship between cytokines was also assessed for each age group (Fig. 1). All cytokines were positively correlated with each other in all age groups. These associations were all significant in children and adolescents ($p < 0.05$). A similar pattern was also observed in adolescents, except IL-10, which was not significantly correlated with other cytokines. Some cytokines were significantly correlated with each other in older adults.

3.3. Baseline cytokine levels and antibody concentrations after booster vaccination

Negative correlations with most baseline cytokines and post-vaccination antibody concentrations were found in children. In children, baseline IFN-γ, IL-2, IL-10, IL-17 A, and IL-17F were negatively correlated with anti-PT IgG and IgA day 28 (Table 4). IL-10 was negatively correlated with PTNA levels on day 28. Baseline IL-2 and IL-10 were negatively correlated with day 28 anti-FHA IgG, while baseline IL-2 and IL-17F were negatively correlated with anti-Prn IgG at day 28. Lastly, baseline IL-5, IL-10, and IL-13 were negatively correlated with anti-DT concentrations at days 28 and 365. As for other age groups, some significant but mainly positive correlations between cytokines and post-vaccination antibody concentrations were observed (Table 4). In adolescents, baseline IL-2 was correlated with day 28 anti-FHA IgG levels, IL-5 and IL-13 with day 365 anti-TT levels, and IL-10 with day 365 anti-Prn IgG levels. For young adults, baseline IFN-γ and IL-5 were correlated with anti-DT concentrations Aat day 28; IL-13 with anti-Prn IgA at day 365 and anti-DT at day 28; and IL-17F with anti-FHA IgG levels at day 28. Correlation analysis was also carried out using only detectable baseline cytokines, which resulted in primarily negative correlations in children and older adults and positive correlations in adolescents and young adults (Supplementary Table 1). These results aligned with the correlations with detectable and undetectable cytokine concentrations.

A repeated measures mixed model was used to confirm the observed

Table 2
The proportion (%) of detectable and undetectable cytokines before vaccination*.

	Children		Adolescents		Young Adults		Older Adults		p-value	Adjusted p-value
	Yes	No	Yes	No	Yes	No	Yes	No		
IFN-γ	44.4	55.6	62.2	37.8	40.0	60.0	39.1	60.9	0.21	1.00
IL-2	36.1	63.9	27.0	73.0	16.0	84.0	17.4	82.6	0.24	1.00
IL-5	58.3	41.7	37.8	62.2	36.0	64.0	26.1	73.9	0.71	1.00
IL-10	58.3	41.7	48.6	51.4	28.0	72.0	21.7	78.3	0.013	0.27
IL-13	58.3	41.7	48.6	51.4	36.0	64.0	39.1	60.9	0.30	1.00
IL-17 A	47.2	52.8	43.2	56.8	36.0	64.0	17.4	82.6	0.094	1.00
IL-17F	58.3	41.7	37.7	62.3	44.0	56.0	30.4	69.6	0.15	1.00

* "Yes" indicates detectable cytokine concentration at baseline; "No" indicates undetectable cytokine concentration at baseline. The p-values were calculated with the Chi-squared test and were adjusted with Bonferroni correction. All adjusted *p*-values of more than 1 is now reported as 1.

Table 3
The concentrations (pg/mL) of baseline cytokines in different age groups. Median [IQR] concentrations are presented*.

	Children	Adolescents	Young Adults	Older Adults	p-value	Adjusted p-value
IFN-γ	0.90 [0.90–50.02]	3.14 [0.90–10.21]	0.90 [0.90–12.49]	0.90 [0.90–5.17]	0.053	1.00
IL-2	2.55 [2.55–12.07]	2.55 [2.55–4.41]	2.55 [2.55–2.55]	2.55 [2.55–2.55]	0.044	0.92
IL-5	2.58 [0.6–32.25]	0.60 [0.60–20.19]	0.60 [0.60–13.33]	0.60 [0.60–1.64]	0.130	1.00
IL-10	0.64 [0.15–7.57]	0.15 [0.15–10.79]	0.15 [0.15–1.67]	0.15 [0.15–0.15]	0.057	1.00
IL-13	30.75 [1.20–326.01]	1.20 [1.20–172.58]	1.20 [1.20–127.94]	1.20 [1.20–68.28]	0.331	1.00
IL-17 A	1.05 [1.05–20.51]	1.05 [1.05–8.61]	1.05 [1.05–6.31]	1.05 [1.05–1.05]	0.178	1.00
IL-17F	85.00[4.50–385.00]	4.50 [4.50–120.00]	4.50 [4.50–120.00]	4.50 [4.50–30.00]	0.157	1.00

* The p-values were calculated using the Kruskal-Wallis test. P-values were adjusted with Bonferroni correction. All adjusted p-values of more than 1 is now reported as 1.

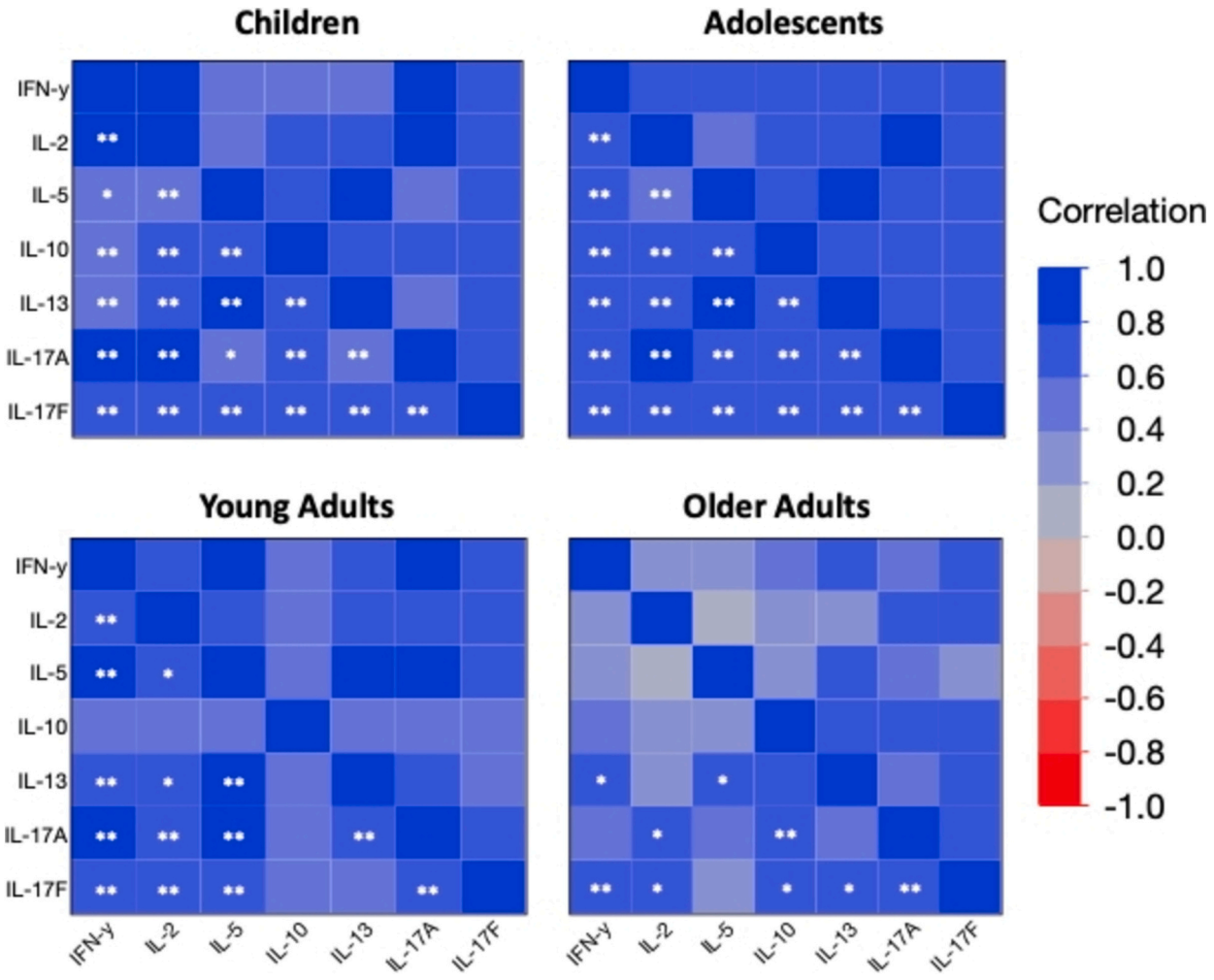

Fig. 1. Heatmap of Spearman's correlations of cytokines (pg/μl) in children, adolescents, young adults, and older adults. All *p*-values were adjusted with Bonferroni correction. * $p < 0.05$, ** $p < 0.01$.

correlations between baseline cytokines and antibody concentrations post-vaccination while taking into account other factors that might affect vaccine responses. Pooled antibody concentrations of all time points showed that children with undetectable baseline cytokines to IFN-γ, IL-2, IL-10, IL-17 A, and IL-17F had significantly higher anti-PT IgG, IgA, and PTNA concentrations (Fig. 2, Table 5). Although the antibody concentration differences between detectable and undetectable baseline cytokine criterion groups did not significantly change over time, except for IL-10 on anti-PT IgA levels ($p = 0.018$, $p_{adj} = 0.13$), the trend where children with undetectable baseline cytokines had higher antibody concentrations over time was still observed. In line with this, children with undetectable IL-10 at baseline had significantly higher anti-PT IgA concentrations at D28 than those with detectable levels ($p = 0.001$, $p_{adj} = 0.007$, MD = 0.36[0.18–0.72]) (Fig. 2 C). Similarly, higher anti-DT IgG concentrations were significantly associated with undetectable IL-5, IL-10, and IL-13 levels at baseline (Fig. 3, Table 5). This was also true for baseline IFN-γ and anti-TT concentrations.

On the other hand, the baseline cytokine detection factors that were significant in the older age groups displayed an opposite trend, where subjects with undetectable baseline cytokines had lower antibody concentrations. Adolescents with undetectable IFN-γ, IL-10, IL-13, and IL-17 A at baseline had significantly lower anti-TT IgG concentrations (Fig. 4, Table 5). This was observed in young adults with IFN-γ, IL-5, IL-13, IL-17 A, and IL-17F for anti-FHA IgA, and IL-13 for anti-Prn IgA

Table 4
Baseline cytokines are negatively correlated with antibody responses after Tdap booster in children but are positively correlated with antibody responses in adolescents and young adults*.

Baseline Cytokine	Antibody Response	Spearman Correlation (r_2)	p-value	Adjusted p-value
Children				
IFN-γ	Anti-PT IgG D28	−0.332	0.048	1.00
	Anti-PT IgA D28	−0.510	0.002	0.39
IL-2	Anti-PT IgG D28	−0.382	0.023	1.00
	Anti-PT IgA D28	−0.529	0.001	0.20
	Anti-FHA IgG D28	−0.335	0.046	1.00
	Anti-Prn IgG D28	−0.439	0.007	1.00
IL-5	Anti-DT D28	−0.422	0.010	1.00
	Anti-DT D365	−0.522	0.001	0.20
IL-10	Anti-PT IgG D28	−0.442	0.007	1.00
	Anti-PT IgA D28	−0.529	0.001	0.20
	PTNA D28	−0.491	0.002	0.39
	Anti-FHA IgG D28	−0.436	0.008	1.00
IL-13	Anti-DT D28	−0.458	0.005	0.98
	Anti-DT D365	−0.471	0.004	0.78
	Anti-DT D28	−0.413	0.012	1.00
	Anti-DT D365	−0.481	0.004	0.78
IL-17 A	Anti-PT IgG D28	−0.348	0.037	1.00
	Anti-PT IgA D28	−0.442	0.007	1.00
IL-17F	Anti-PT IgG D28	−0.421	0.011	1.00
	Anti-PT IgA D28	−0.411	0.013	1.00
	Anti-Prn IgG D28	−0.417	0.011	1.00
Adolescents				
IL-2	Anti-FHA IgG D28	−0.3259	0.049	1.00
IL-5	Anti-TT D365	0.3326	0.044	1.00
IL-10	Anti-Prn IgG D365	0.3633	0.027	1.00
IL-13	Anti-TT D365	03299	0.046	1.00
Young Adults				
IFN-γ	Anti-DT D28	0.4262	0.034	1.00
IL-5	Anti-DT D28	0.5204	0.0077	1.00
IL-13	Anti-Prn IgA D365	0.4261	0.034	1.00
	Anti-DT D28	0.4236	0.035	1.00
IL-17F	Anti-FHA IgA D28	0.4036	0.045	1.00

* All p-values were adjusted with Bonferroni correction. All adjusted p-values of more than 1 is now reported as 1.

(Fig. 5, Table 6). Moreover, the interaction with baseline IL-13 detection and time for anti-FHA IgA was significant ($p = 0.018$, $p_{adj} = 0.13$), and baseline antibody concentrations between detectable and undetectable IL-13 levels differed ($p = 0.014$, $p_{adj} = 0.98$, MD = 0.36[0.18–0.72]). While a similar trend was observed for PTNA concentrations in older adults with significant baseline detection cytokines and time interaction for IL-2 ($p = 0.018$, $p_{adj} = 0.13$), IL-10 ($p = 0.013$, $p_{adj} = 0.091$), IL-13 ($p = 0.008$, $p_{adj} = 0{,}056$), IL-17 A ($p = 0.021$, $p_{adj} = 0.15$), and IL-17F ($p = 0.033$, $p_{adj} = 0.23$), no significant differences were observed between detectable and undetectable cytokines at each time point (results not shown).

4. Discussion

Adequate responses to vaccine antigens are essential for protection against pertussis. However, the intrinsic factors contributing to hyporesponsiveness to pertussis vaccination are poorly understood. In this study, longitudinal serum samples collected before, one month, and one year after the Tdap booster vaccination were studied in four age groups of Finnish participants to assess how baseline cytokines influence antibody concentrations to vaccine antigens and PTNAs. Several baseline cytokines were found to negatively correlate with anti-PT antibody concentrations (IgG and IgA) in children, and the undetectable levels of these cytokines before vaccination were associated with higher anti-PT antibody levels, as well as anti-TT and anti-DT IgG. Meanwhile, opposite trends were observed in older age groups, with several baseline cytokines having positive correlations with anti-TT IgG in adolescents and anti-FHA IgA in young adults, showing that undetectable levels of these cytokines at baseline were associated with lower antibody concentrations. To our knowledge, this is the first study to show that the association of baseline cytokines on antibody concentrations to pertussis booster vaccination is age-dependent and antigen-specific.

The biological mechanisms underlying the influence of baseline IFN-γ, IL-5, IL-13, IL-17 A, and IL-17F concentrations on the initiation and longevity of pertussis vaccine-specific antibody responses remain unclear. In addition to the recruitment of CD4 and CD8 T cells, and natural killer (NK) cells at the site of infection, IFN-γ modulates the differentiation of B cells. Studies in healthy adult PBMCs stimulated with pokeweed mitogen have shown that the addition of IFN-γ to unstimulated PBMCs as a control suppresses spontaneous IgG1 secretion, a primary constituent of total IgG, while elevating IgG2 levels without significantly impacting IgG3 or IgG4 production levels [12]. The presence of IFN-γ derived from Th cells or NK cells before vaccination can be attributed to exposure to external or internal factors, such as infection. Therefore, the presence of IFN-γ at baseline may decrease antibody concentrations after an aP booster that was observed in children. However, baseline IFN-γ was positively associated with anti-TT antibody concentrations in children and adolescents and with anti-FHA IgA in young adults, an opposite effect from what was observed with anti-PT antibody levels in children. Studies suggest that IFN-γ has a dual stimulatory and inhibitory effect on B cells that is dependent on the type of stimulant and the state of B cell activation [13,14]. Similar results with pokeweed mitogen-stimulated and -unstimulated PBMCs were observed in LPS-stimulated resting B cells from mice [15]. On the other hand, healthy adults vaccinated with a malaria sporozoite vaccine conjugated to TT in addition to the administration of recombinant human IFN- γ showed that whole blood stimulation with TT resulted in increased lymphocyte proliferation, which was not observed when stimulated with the vaccine alone [16]. This indicates that in addition to age, as this effect on anti-PT antibody concentrations in the older age groups was not observed, the influence of baseline IFN-γ may be antigen-specific. Moreover, it should be kept in mind that almost all participants in the study had pre-existing TT antibodies (97 % of children, 95 % of adolescents, 100 % of young adults, and 100 % of older adults had anti-TT IgG concentrations concentrations>0.1 IU/mL), suggesting that the memory to this antigen may be different from that to PT (83 % of children, 78 % of adolescents, 40 % of young adults, and 70 % of older adults had anti-PT IgG concentrations >5 IU/mL).

Regarding other cytokines, it is known that IL-5 induces B-cell survival, proliferation, and differentiation into antibody-secreting plasma cells [17,18]. In contrast, IL-13 induces total IgG and IgG4 synthesis in immature human B cells [19,20]. IL-17A and IL-17F are Th17 pro-inflammatory cytokines with high homology; the former aids B-cell activation and proliferation by influencing germinal center formation and isotype class switching, while the latter may play a role in mouse germinal center formation [21–25]. These observations were in agreement with our results in the older age groups. In adolescents, undetectable IL-13 and IL-17A at baseline were linked to lower anti-TT IgG.

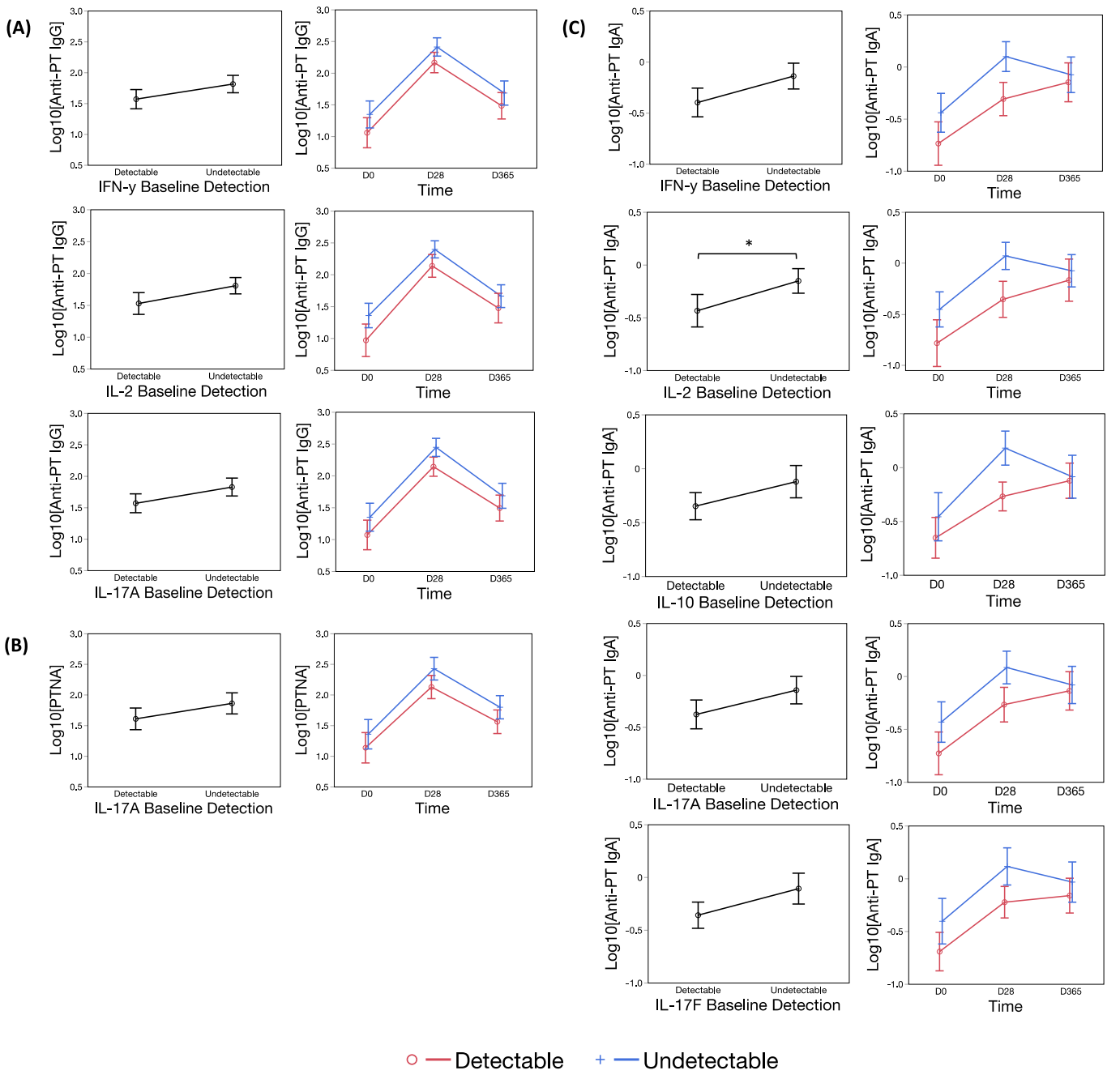

Fig. 2. Effect of baseline cytokines on anti-PT IgG (A), PTNA (B), and anti-PT IgA (C) responses in children. Images on the left show cytokine baseline detection factor with pooled antibody data of all three time points. Images on the right show the interaction with cytokine baseline detection with time. * $p < 0.05$.

In young adults, undetectable baseline IL-5, IL-13, IL-17A, and IL-17F were associated with lower anti-FHA IgA concentrations and IL-13 with anti-Prn IgA. However, age seems to play a role in determining the impact of baseline cytokines on antibody concentrations, as our results suggest that baseline IL-5, IL-13, IL-17A, and IL-17F appear to have an inhibitory link on antibody levels in children after an aP booster. This dichotomous phenomenon was observed in other vaccines as well. Although opposite to this study, a multi-cohort study involving influenza vaccines revealed that enriched pro-inflammatory gene signatures at baseline positively correlated with antibody responses in individuals under 35 but appeared to correlate negatively with antibody responses in those over 65 [26].

This age-dependent relationship was observed in IL-2 and IL-10 cytokines in this study. IL-2 is secreted by activated T cells with a dual role in T and NK cell proliferation [27], while IL-10 is an anti-inflammatory cytokine that suppresses the activities of antigen-presenting cells, leading to reduced B-cell stimulation [28]. An influenza infection model in mice showed that IL-2 impairs influenza-specific germinal centers, consequently diminishing antibody responses through its inhibitory role on germinal center T follicular helper cell formation and maintenance [29,30]. This was in line with the results in children where undetectable IL-2 levels at baseline were associated with higher anti-PT IgG and IgA levels. This study found no significant associations with antibody concentrations in the older age groups despite high frequencies of undetectable IL-2 in all age groups. Regarding IL-10, a study on healthy adults given malaria vaccines indicated a negative correlation between baseline IL-10 and antibody levels post-vaccination [31]. Again, this negative correlation was observed in children with undetectable IL-10,

Table 5
Mean differences in antibody responses between detectable and undetectable cytokines for all age groups*.

Baseline Cytokine	Antibody Response	Mean Difference (95 % CI)	p-value	Adjusted p-value
Children				
IFN-γ	Anti-PT IgG	0.57 (0.35–0.92)	0.023	0.16
	Anti-PT IgA	0.55 (0.36–0.85)	0.009	0.06
	Anti-TT	0.43 (1.06–2.29)	0.026	0.18
IL-2	Anti-PT IgG	0.53 (0.32–0.86)	0.012	0.08
	Anti-PT IgA	0.52 (0.33–0.81)	0.005	0.04
IL-5	Anti-DT	0.43 (0.22–0.83)	0.013	0.09
IL-10	Anti-PT IgA	0.59 (0.38–0.93)	0.024	0.17
	Anti-DT	0.50 (0.25–0.99)	0.044	0.31
IL-13	Anti-DT	0.45 (0.23–0.84)	0.014	0.10
	Anti-PT IgG	0.55 (0.34–0.89)	0.016	0.11
IL-17 A	Anti-PT IgA	0.58 (0.38–0.91)	0.018	0.13
	PTNA	0.56 (0.32–0.98)	0.044	0.31
IL-17F	Anti-PT IgA	0.56 (0.36–0.87)	0.012	0.08
Adolescents				
IFN-γ	Anti-TT	1.59 (1.06–2.41)	0.027	0.19
IL-10	Anti-TT	1.52 (1.01–2.27)	0.043	0.30
IL-13	Anti-TT	1.55 (1.04–2.32)	0.032	0.22
IL-17 A	Anti-TT	1.59 (1.06–2.37)	0.025	0.18
Young Adults				
IFN-γ	Anti-FHA IgA	7.20 (1.55–33.46)	0.014	0.10
IL-5	Anti-FHA IgA	6.82 (1.39–33.44)	0.020	0.14
IL-13	Anti-FHA IgA	7.33 (1.52–35.36)	0.015	0.11
	Anti-Prn IgA	5.04 (1.42–17.94)	0.015	0.11
IL-17 A	Anti-FHA IgA	6.04 (1.20–30.40)	0.031	0.22
IL-17F	Anti-FHA IgA	8.25 (1.89–36.08)	0.007	0.05

* Pooled antibody concentrations are in IU/mL except for PTNA and were logarithmically transformed. The p-values were calculated using a repeated-measures mixed model with Tukey's HSD correction and were adjusted with Bonferroni correction.

which was associated with higher anti-PT IgA and -DT IgG concentrations but was positively associated with anti-TT IgG in adolescents. Further studies are required to understand this dichotomous phenomenon.

Despite this age-dependent association of baseline cytokines with antibody concentrations, analysis of the proportion of detectable and undetectable cytokines and their concentrations did not show significant differences between age groups. However, correlation analysis of cytokines with one another revealed that all cytokines were significantly positively correlated with each other in children and adolescents but were less correlated in young and older adults. This may indicate an age-dependent cytokine cascade where cytokines have a higher influence on each other's activities in younger age groups but less in the older age groups. Thus, the observed age-related differences could be attributed to this age-dependent cytokine cascade in addition to other intrinsic immune factors that alter the activity of baseline cytokines on vaccine concentrations. For instance, in this study, all children were primed by DTaP vaccine, whereas half of adolescents and all adults were primed by wP. Therefore, it is possible that the observed association of baseline cytokines with antibody responses after Tdap booster vaccination might be linked to the priming effect by acellular vaccines.

This study showed that in children, baseline IFN-γ, IL-2, IL-10, IL-17A, and IL-17F were associated with anti-PT IgA. However, it is important to note that IgA concentrations only slightly increase with pertussis vaccination, especially in children, and that the concentration and the magnitude of IgA responses increase with age (Supplementary Fig. 1) [5]. Specific IgA responses arise following an individual's exposure to *B. pertussis* during their lifetime and are activated via the mucosal route [32]. Even though we observed this significance and saw the same trend in kinetics in this study, IgA concentrations in children were already low even after vaccination. Moreover, although the older age groups had generally higher anti-PT IgA concentrations, baseline cytokines were only associated with anti-FHA IgA but not other antigens. These results should be interpreted with caution.

Although our findings support that pre-existing cytokines may contribute to hypo-responsiveness after an aP booster in children, this study has limitations. First, the sample size in each age group was small, which may contribute to insufficient statistical power for post-hoc analyses. Our study reported several significant baseline detection factors associated with pooled antibody levels of all three time points in different age groups. However, when analyzing the interaction of this cytokine baseline detection factor with time, we could not identify significant differences in antibody concentrations between detectable and undetectable cytokines at each time point. In addition to this, we did not observe significant differences in PTNA concentrations in older adults between detectable and undetectable cytokines despite the baseline detection and time factor being significant. Moreover, most of the *p*-values did not survive Bonferroni correction. Therefore, our findings are not generalizable to the broader population and need validation in a

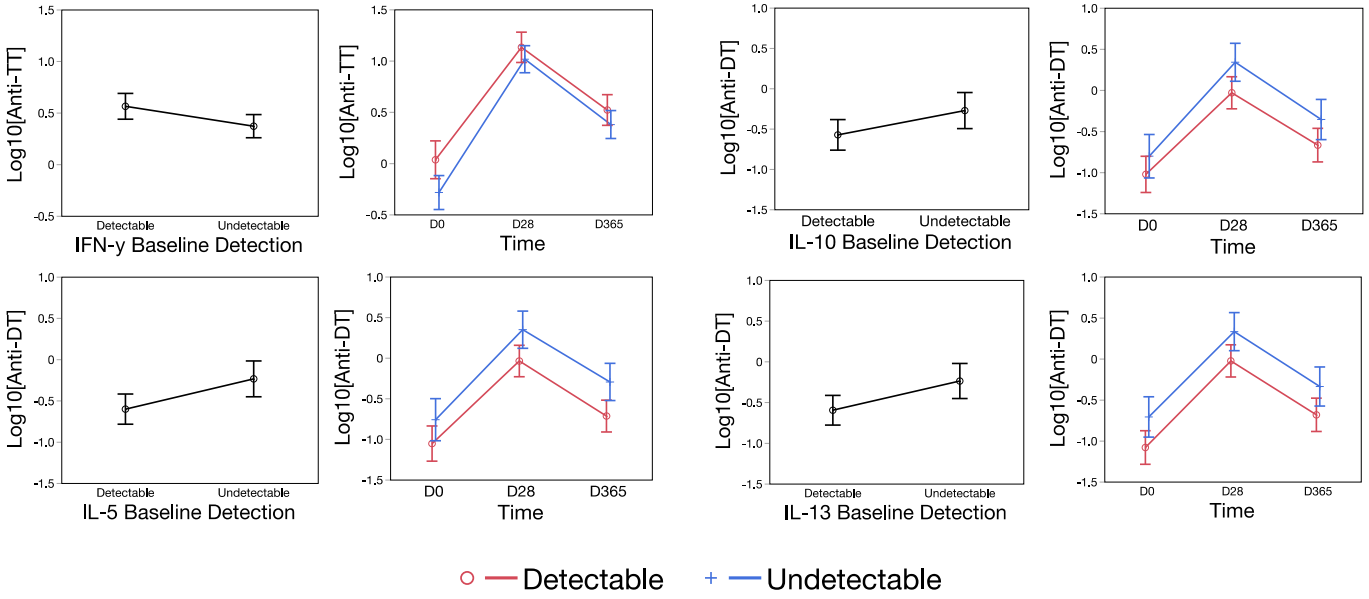

Fig. 3. Effect of baseline IFN-γ on anti-TT responses, and IL-5, IL-10, and IL-13 on anti-DT responses in children. Images on the left show cytokine baseline detection factor with pooled antibody data of all three time points. Images on the right show the interaction with cytokine baseline detection with time.

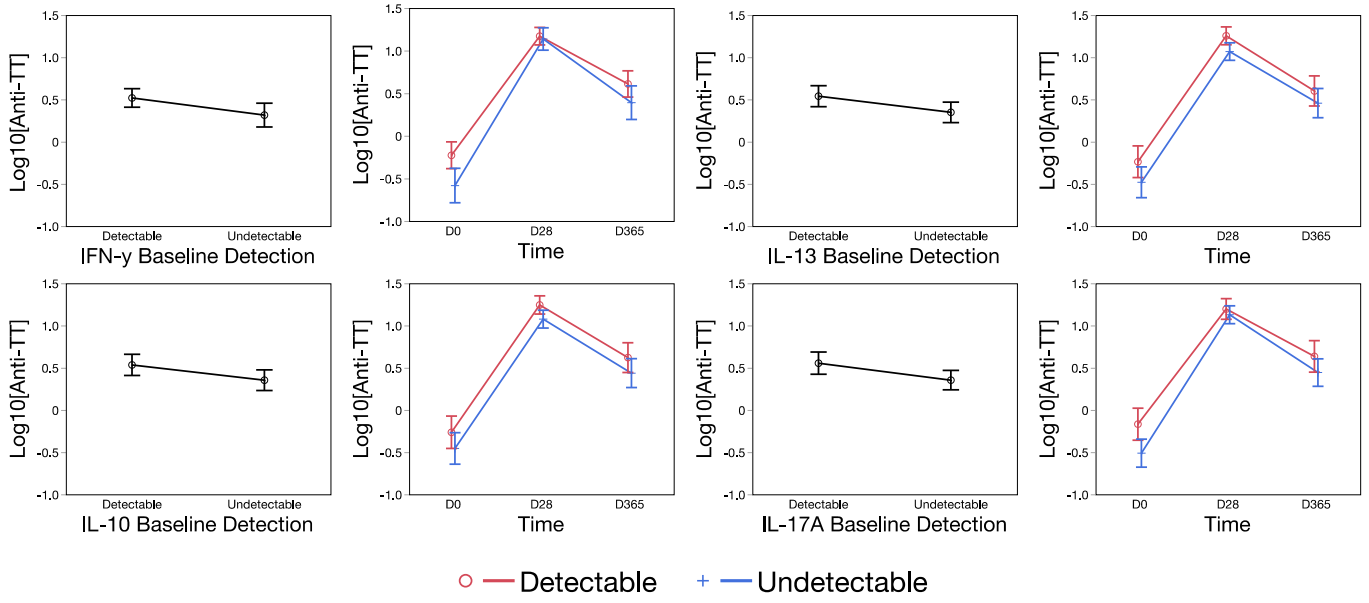

Fig. 4. Effect of baseline IFN-γ, IL-10, IL-13 and IL-17 A on anti-TT responses in adolescents. Images on the left show cytokine baseline detection factor with pooled antibody data of all three time points. Images on the right show the interaction with cytokine baseline detection with time.

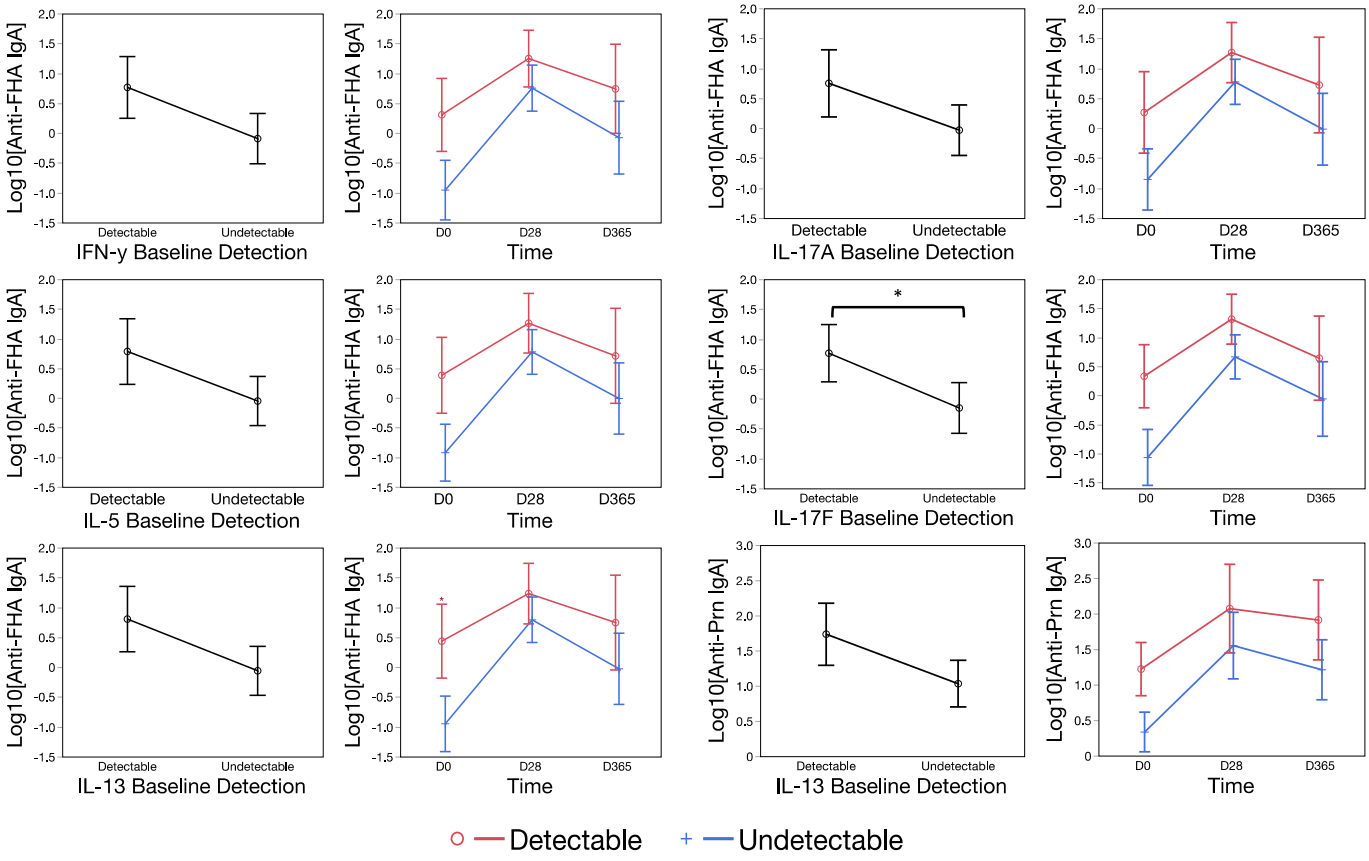

Fig. 5. Effect of baseline, IFN-γ, IL-5, IL-13, IL-17 A, and IL-17F on anti-FHA IgA responses, and IL-13 on anti-Prn IgA responses in young adults. Images on the left show cytokine baseline detection factor with pooled antibody data of all three time points. Images on the right show the interaction with cytokine baseline detection with time. * $p < 0.05$.

larger, independent cohort. Second, although a broad range of cytokines was selected that represent Th1, Th17, Th2, and Treg cytokines known to affect antibody responses, these only represent a subset of immune signaling pathways. A larger sample size would allow further analysis of other immune factors that contribute to vaccine response through multi-omic approaches utilized in other systems vaccinology-related research by building a more complex model that would consider all these factors [33]. Third, the cytokine concentrations measured in this study from unstimulated sera were generally low. However, other studies with similar methods also reported low or undetectable concentrations of

cytokines [34]. Fourth, the gender distribution in adult age groups was imbalanced, with a higher proportion of female subjects, which was primarily due to the failure to meet the inclusion criteria of not having received a booster vaccine that contains pertussis in the last five years [5]. In Finland, males aged 18 and above entering military service usually receive Tdap booster vaccinations. It is known that there are sex differences in innate and adaptive immunity, and the possible effects of this bias on the results cannot be excluded [35]. Despite this, analysis of the sex effects as a fixed factor in the model on antibody responses after booster was insignificant [5]. Fifth, the pubertal stage is also known to influence cytokine levels in adolescents, and we cannot rule out the potential impact of this bias on our findings [36]. Lastly, the proposed mechanistic explanations for these observations should be further studied.

Despite these limitations, our findings further support the possible relationship between pre-existing cytokine concentrations and pertussis booster vaccine concentrations. As previously discussed, our study revealed age-dependent links between baseline cytokine levels and post-vaccination antibody concentrations. Age-dependent relationships with baseline immune profiles were also observed in responses to other vaccines, such as influenza, varicella zoster, and hepatitis B [9,26,37]. Barring further validation, specific cytokines associated with low or high antibody concentrations could be used as a predictive biomarker, along with other immunological factors associated with pertussis vaccination responses, for personalized vaccination strategies. However, an internationally accepted correlate of protection against pertussis currently does not exist, making it difficult to gauge whether a person is a low- or high-responder after vaccination. A better understanding of the underlying mechanisms behind pertussis immunity will pave the way to establishing personalized vaccination.

In conclusion, the results of this exploratory study indicate a potential role of pre-existing cytokines in children who receive pertussis booster vaccine and warrant further studies in different populations. However, the results should be interpreted with caution as the number of subjects is limited.

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CRediT authorship contribution statement

Denise Anabe: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Johanna T. Teräsjärvi:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Alex-Mikael Barkoff:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Aapo Knuutila:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Bernd Pape:** Writing – review & editing, Methodology, Data curation. **Pieter van Gageldonk:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Annemarie Buisman:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Jussi Mertsola:** Writing – review & editing, Investigation, Funding acquisition, Data curation. **Qiushui He:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2024.126573.

Data availability

The data that has been used is confidential.

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