Archives of Physiology and Biochemistry, 2012; 118(4): 197–203

ISSN 1381-3455 print/ISSN 1744-4160 online
DOI: 10.3109/13813455.2012.659669

informa
healthcare

ORIGINAL ARTICLE

Influence of an immunopotentiator Polyoxidonium on cytokine profile and antibody production in children vaccinated with Priorix

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Abstract

60 children aged 1-2 years old (32 boys and 28 girls) were vaccinated with Priorix. Vaccinated children included healthy control (19 children, group 1), and children with immunological disturbances such as episodes of respiratory infection. From the latter group, 20 children did not receive (group 2), and 21 children received 0.15 mg/kg of Polyoxidonium simultaneously with the vaccine (group 3). On days 7 and 30 after vaccination, CD-markers on lymphocytes and concentration of specific antibodies, as well as levels of 11 cytokines in serum were evaluated by flow cytometry, ELISA, and multiplex techniques respectively. It was found that injection of Polyoxidonium skewed T helper differentiation to Th2 type. Antibody responses were significantly higher in children with preferable Th2 responses. Children from group 3 possessed higher titers of specific IgG-antibodies. Our study shows that Polyoxidonium could smooth out the immune reaction on vaccination. It is important for children with some immunological disturbances.

Keywords: Measles-mumps-rubella vaccination, cytokines, Th1/Th2 skewing, Polyoxidonium, Bio-Plex

Introduction

Measles, mumps and rubella are highly infectious viral diseases that may cause serious harm to children. In spite of the worldwide program of two-doses measles-mumps-rubella vaccination, measles remains a cause of 197,000 fatal cases each year (Centers for Disease Control and Prevention, 2007) and 100,000 infants worldwide develop congenital rubella syndrome (Robertson *et al.*, 2003). Measles outbreaks can occur even in highly vaccinated countries such as USA, Canada, Austria, Italy, Germany, France, Britain and many others (Hickman *et al.*, 2011). Moreover, measles infection may occur in vaccinated individuals (Rota *et al.*, 2011). It is clear that macro-organism's status plays a pivotal role in vaccine response. Vaccine virus immunization induces both $CD4^+$ and $CD8^+$ T-cells decreasing (Rager-Zisman *et al.*, 2003; Ovsyannikova *et al.*, 2003a). Interferon-γ (IFN-γ) and interleukin 4 (IL-4) are Th1 and Th2 cytokines, respectively. Immunization affects Th1/Th2 cell differentiation, IgE production, and macrophage stimulation (Seder and Paul, 1994). Preferential activation of IL-4 producing Th2 cells by measles vaccine has been reported (Ward and Griffin, 1993). However, it has been shown that Th1 cytokine production was activated by measles virus after the vaccination (Ovsyannikova *et al.*, 2003b). Later it has been determined that both Th1 and Th2 types of immune responses could be realized after a MMR vaccination (Dhiman *et al.*, 2005). On the other hand a progressive decrease was found in specific antibody levels in all vaccinees 10 years after the second dose of MMR inoculation (Hickman *et al.*, 2011). Some pharmaceuticals with the immunopotentiation effect may modulate the number of lymphocytes and their functions. An immunomodulating agent Polyoxidonium, which is a polyion immunostimulant (Dyakonova *et al.*, 2004), has been used in immunocompromised patients treatment. Immunostimulatory activity of Polyoxidonium was also confirmed in clinical trials as a component of an influenza vaccine Grippol® (Petrov *et al.*, 1985) and a live brucellosis vaccine (Denisov *et al.*, 2010).

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(Received 10 December 2011; revised 12 January 2012; accepted 17 January 2012)

The aim of this study was to investigate the influence of the Polyoxidonium injection on the immune response against trivalent live attenuated measles, mumps and rubella vaccine Priorix.

Methods

Study subjects

Sixty children aged 1 to 2 years old (32 boys and 28 girls) were vaccinated with trivalent live attenuated measles, mumps and rubella vaccine Priorix® (GlaxoSmithKline, Belgium). Vaccinated children included healthy control (19 children, group 1), and children with immunological disturbances such as episodes of respiratory infection, bronchitis, and/or otitides (41 children). From the latter group, 20 children did not receive (group 2), and 21 children did receive 0.15 mg/kg of Polyoxidonium, according to the manufacturer's instructions, simultaneously with the vaccine (group 3). Written informed consent was obtained from each parent for this study. The study was approved by Ethics Committee of the G.N. Gabrichevsky Institute of Epidemiology and Microbiology.

Materials

Polyoxidonium® (Petrovax Pharm, Moscow, Russia) is a polyion immunostimulant (N-oxidized polyethylene-piperazine derivative) (Nekrasov and Puchkova, 2002) used in immune-compromised patients treatment.

Priorix® is a trivalent live attenuated measles-mumps-rubella vaccine (GlaxoSmithKline, Belgium) containing the Schwartz strain of measles virus ($\geq 10^3$ 50% tissue culture infective doses [$TCID_{50}$]), the RIT 4385 a derivative of Jeryl Lynn strain of mumps virus ($\geq 10^3$ $TCID_{50}$), and the Wistar RA 27/3 strain of rubella virus ($\geq 10^3$ $TCID_{50}$).

Blood collection

Venous blood samples (3 ml) were collected in (a) serum separator tubes (sera were separated by centrifugation at 900 × g for 15 min at room temperature, aliquoted, stored at −70°C, and used within 100 days) and (b) heparinized Vacutainer tubes (2 ml) for peripheral blood mononuclear cells (PBMC) isolation. Blood was diluted twice the original volume with phosphate-buffered saline (PBS), pH 7.4 and peripheral blood mononuclear leukocytes were isolated by Ficoll-Hypaque (Amersham Pharmacia Biotech, Piscataway, NJ) density gradient centrifugation. These freshly isolated PBMC were used for the CD-marker detection. Blood samples were collected before vaccination and then again one week and one month later.

FACS analysis

Phenotype of blood lymphocytes was identified by flow cytofluorimetry using Becton-Dickinson's technology and materials. The PBMC samples in PBS (100 µl) were treated with 20 µl fluorescent-labelled monoclonal antibodies according to the manufacturer's instructions and incubated for 30 min on ice in the dark. For counting number of cell populations we used three-colour staining (anti-CD3-Per-CP, anti-CD4-FITC, anti-CD8-PE, or anti-CD3-FITC, anti-CD16/56-PE, anti-CD19-Per-CP). Antibodies were obtained from BD Biosciences. After incubation cells were washed twice in PBS. After final re-suspension samples were analysed immediately. Flow cytometric analysis was performed by FACSCalibur with CellQuest software (Becton-Dickinson, Franclin Lakes, NJ, USA). Lymphocytes were identified after gaiting on $CD45^+$cells, and 10,000 events were acquired for each measurement. The data are presented as absolute number of respective cells in microlitre.

Cytokine assay

Cytokine concentrations were estimated by use of multiplex technology (BioRad, USA) with commercial kit (BioRad, USA) using the high sensitivity protocol according to the manufacturer's instructions. The 11-bead mixture with distinct fluorescence intensities coated with capture antibodies specific for IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IFN-γ and tumour necrosis factor-α (TNF-α) was added to a 96-well filter plate, followed by serum samples or standard dilutions. The plate was incubated at room temperature for 60 min in the dark and then washed. Then a mixture of biotinylated detection antibodies was added. After 30 min incubation and washing streptavidin-phycoerythrin conjugate was added. The data were analysed by BioPlex suspension array system using BioPlex Manager software (BioRad, USA). Cytokine concentrations in each tested sample were automatically calculated using a standard curve. Assay sensitivity was from 0.2 to 3200 pg/ml.

Antibody assay

Specific IgM and IgG antibodies against rubella and mumps were measured using ELISA kits (Human, Wiesbaden, Germany) according to the manufacturer's instructions. Antimeasles IgM and IgG were tested using ELISA kit (Euroimmun, Lübeck, Germany). IgE antibodies were tested with enzyme-linked immunosorbent assay with commercially kit (DIAplus, Moscow, Russia) according to the manufacturer's instructions.

Statistical analysis

A χ^2 test was used to analyse distribution types and verify hypotheses. The two-sided *t*-test or the non-parametric Wilcoxon test were employed to compare the means of two groups. Values of $p < 0.05$ were considered significant. Differential calculus was used for investigation the dynamic of cytokines concentration changing (Bermant and Aramantovich, 2003). We calculated the second derivative from cytokine concentration function ($f''(x)$). Negative or positive $f''(x)$ corresponds to the direction

and type of immune response. The detailed protocol has been described elsewhere (Toptygina *et al.*, 2005). Pearson's correlation coefficients were calculated to assess association of cytokines.

Results

Lymphocyte subsets

Absolute numbers of lymphocytes subsets obtained before and after vaccination are presented in Table 1. On the seventh day after vaccination, children from the group 1 had decreased average total numbers of lymphocytes and each subset compared to pre-vaccination levels ($p < 0.05$). These characteristics were recovered to the normal ranges within a month after the vaccine inoculation. In group 2 we did not observe such changes in lymphocyte count. In the latter group, the numbers of total lymphocyte and T cell subsets tended to increase 7 days after immunization ($p > 0.05$). Moreover, one month after vaccination the characteristics of lymphocyte subsets in group 2 did not significantly differ from those of group 1. Interestingly, that children from group 3 that received Polyoxidonium demonstrated responses similar to group 1 (healthy control). Differences between children from group 2 and group 3 in total numbers of lymphocytes and each subset on the seventh day after vaccination were significant ($p < 0.01$). These data demonstrate an early immunomodulating effect of Polyoxidonium that may correct or prevent changing in T lymphocyte count.

Serum cytokines

Summarized data of serum cytokine concentration are presented in Table 2. Average concentrations of serum cytokines did not change significantly in all three groups. Unfortunately, dispersion between individuals disguised cytokines changing. To avoid dispersion due to varying results between samples we used ratio (serum level of IFN-γ to serum level IL-4) for each subject to characterize dominant Th1/Th2 skewing. The function of serum cytokines concentration is a curve looked like a parabola. Usually differential calculus is used for investigation such curve. If the curve has maximum, the second derivative $f''(x)$ is negative. On the contrary, if the curve has minimum, $f''(x)$ is positive. We used differential calculus for investigation of dynamic processes in serum levels of cytokines after vaccine inoculation. If immune response progresses with IFN-γ prevalence, the calculated second derivative is negative and corresponds to Th1 type immune response. Positive $f''(x)$ corresponds to Th2 type response. The analysis of our data showed that in group 1 nine children demonstrated Th1 type response and 10 children showed Th2 type response. Similar distribution was obtained in group 2 (10 children showed Th1 type response and 10 children demonstrated Th2 type). In group 3 such characteristics

Table 1. Effect of measles, mumps and rubella vaccination on numbers of lymphocytes in peripheral blood (absolute lymphocyte count per μl).

	Group 1			Group 2			Group 3		
	Before vaccination	Day 7*	Day 30*	Before vaccination	Day 7*	Day 30*	Before vaccination	Day 7*	Day 30*
Lymphocytes	5258 ± 273	3492 ± 318**	4931 ± 349	3084 ± 254****	3722 ± 311	4695 ± 337**	3383 ± 283****	3022 ± 291***	4110 ± 347
CD3+	3768 ± 264	2385 ± 259**	3501± 315	2086 ± 247****	2670 ± 255	3146 ± 305**	2308 ± 256****	2096 ± 243***	2626 ± 271
CD19+	1074 ± 98	697 ± 57**	900 ± 86	612 ± 59****	690 ± 63	807 ± 76**	643 ± 59****	533 ± 49***	770 ± 66
CD16+	656 ± 61	472 ± 43**	663 ± 63	480 ± 45****	512 ± 48	749 ± 69**	440 ± 39****	388 ± 35***	594 ± 53
CD4+	2554 ± 273	1624 ± 184**	2405 ± 223	1295 ± 110****	1720 ± 157	2087 ± 218**	1541 ± 123****	1399 ± 128***	1755 ± 211
CD8+	1233 ± 113	737 ± 65**	1214 ± 115	675 ± 61****	835 ± 79	1037 ± 97**	762 ± 71****	652 ± 62***	915 ± 88

Notes: *Days after vaccine inoculation.
**$p < 0.05$ compared with initial levels using two-sided t-test.
***$p < 0.01$ compared with Group 2 using paired Wilcoxon test.
****$p < 0.01$ compared with Group 1 using two-sided t-test.

Table 2. Cytokine profile in serum of vaccinated children (pg/ml).

	Group 1			Group 2			Group 3		
	Before vaccination	Day 7	Day 30	Before vaccination	Day 7	Day 30	Before vaccination	Day 7	Day 30
IL-1	0.52 ± 0.05	0.48 ± 0.06	0.36 ± 0.05	0.50 ± 0.07	0.49 ± 0.05	0.48 ± 0.05	0.46 ± 0.08	0.57 ± 0.07	0.39 ± 0.06
IL-2	1.47 ± 0.09	1.5 ± 0.13	1.15 ± 0.09	1.52 ± 0.11	1.51 ± 0.12	1.49 ± 0.08	1.54 ± 0.10	1.55 ± 0.15	1.47 ± 0.09
IL-4	2.34 ± 0.19	2.46 ± 0.24	2.34 ± 0.16	2.23 ± 0.25	2.54 ± 0.28	2.47 ± 0.21	2.45 ± 0.31	2,76 ± 0.34	2.63 ± 0.26
IL-5	0.14 ± 0.01	0.16 ± 0.01	0.19 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.18 ± 0.01
IL-6	1.81 ± 0.32	1.63 ± 0.19	1.71 ± 0.05	1.79 ± 0.35	1.61 ± 0.28	1.63 ± 0.25	1.86 ± 0.37	1.78 ± 0.31	1.74 ± 0.28
IL-7	1.61 ± 0.21	1.76 ± 0.18	1.63 ± 0.13	1.65 ± 0.24	1.54 ± 0.22	1.68 ± 0.23	1.63 ± 0.27	1.78 ± 0.26	1.66 ± 0.22
IL-8	1.76 ± 0.15	2.37 ± 0.09	2.23 ± 0.21	1.84 ± 0.21	2.43 ± 0.19	2.47 ± 0.22	1.85 ± 0.22	2.41 ± 0.21	2.38 ± 0.23
IL-10	0.16 ± 0.01	0.22 ± 0.01	0.27 ± 0.02	0.18 ± 0.03	0.24 ± 0.04	0.28 ± 0.03	0.19 ± 0.03	0.17 ± 0.03	0.17 ± 0.02
IL-12	1.04 ± 0.07	1.26 ± 0.08	1.37 ± 0.05	1.15 ± 0.08	1.25 ± 0.09	1.16 ± 0.07	1.20 ± 0,09	1.31 ± 0.10	1.24 ± 0.11
TNF-α	0.41 ± 0.06	0.49 ± 0.03	0.48 ± 0.03	0.43 ± 0.05	0.47 ± 0.07	0.45 ± 0.05	0.43 ± 0.06	0.55 ± 0.08	0.51 ± 0.07
IFN-γ	10.51 ± 0.52	11.59 ± 0.69	9.72 ± 0.36	9.65 ± 0.54	12.31 ± 0.66	10.84 ± 0.57	9.73 ± 0.51	8.67 ± 0.54	9.51 ± 0.52

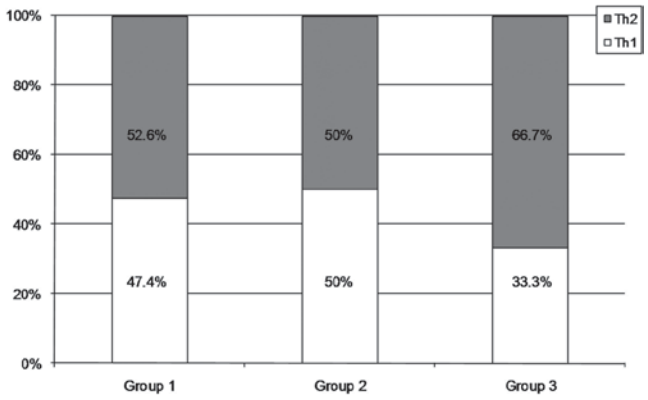

Figure 1. Th1/Th2 distribution of children vaccinated with trivalent live attenuated measles, mumps and rubella vaccine Priorix(f″ (x) criterion). Group 1 includes 19 healthy children, Group 2 includes 20 "immune-compromised" children, they had one or more episodes of respiratory infection and 21 children were simultaneously vaccinated and injected with 0.15 mg/kg of Polyoxidonium (Group 3). The serum samples were collected before, 1 and 4 weeks after vaccination and ratio of IFN- serum level to serum level of IL-4 were determined. Differences in distribution types between Groups 1, 2 and Group 3 are significant ($p < 0.05$ using χ^2 test).

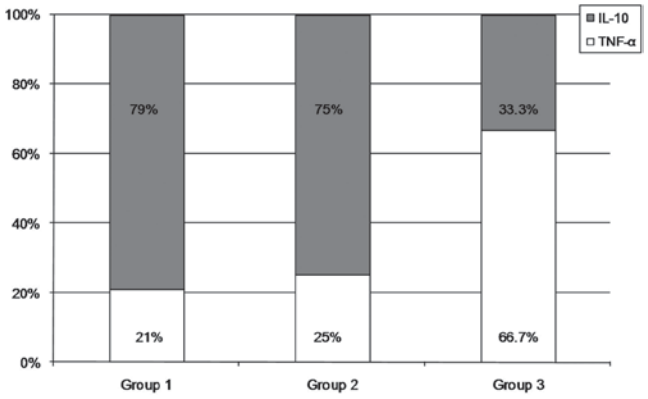

Figure 2. Distribution in TNF- or IL-10 responding children after vaccination with Priorix® (f″(x) criterion). A significant difference in distribution types between Groups 1, 2 and Group 3 were observed ($p < 0.001$ using χ^2 test). See the legend to Figure 1.

showed 7 and 14 individuals, respectively (Figure 1). Type of immune response did not correlate with serum level of cytokines. Differences in distribution types between groups 1 and 3 or groups 2 and 3 were significant ($p < 0.05$).

We used the second derivative calculation for ratio of other pair of cytokine: pro-inflammatory TNF-α and anti-inflammatory IL-10. It was shown that 15 of 19 children of group 1 (79%) and 15 of 20 children of group 2 (75%) demonstrated after vaccination the superiority of anti-inflammatory IL-10 over pro-inflammatory TNF-α. On the contrary, in most subjects of group 3 (14 of 21) a predominance of pro-inflammatory TNF-α was observed (Figure 2). A significant difference in distribution types between groups 1, 2 and group 3 were observed ($p < 0.001$). We conclude that using Polyoxidonium simultaneously with the vaccine lead to skewing the dominant immune responses from Th1 to Th2 type and increasing of pro-inflammatory cytokines, that confirmed immunomodulating properties of Polyoxidonium.

Table 3 shows the correlation between different cytokines changing. IL-7 and IL-10 levels were negatively correlated with other cytokines and demonstrated strong positive correlation with each other ($r = 0.97$). Strong positive correlation was found between IL-1 and IL-6 ($r = 0.76$), IL-2 and IL-6 ($r = 0.91$), IL-2 and IL-4 ($r = 0.89$), IL-4 and IL-6 ($r = 0.82$), IFN-γ and IL-4 ($r = 0.75$), IL-6 and IFN-γ ($r = 0.80$), and IFN-γ and TNF-α ($r = 0.86$).

Antibodies response

All children were sero-negative before the vaccination. Sero-conversion (appearance of specific IgM and IgG antibodies) was reached within one month in 98.5% cases (measles), 95.6% (rubella) and 85.3% (mumps). In group 2 a lower level of specific immune response was detected. Higher titres of the specific antibodies have been determined in children receiving vaccine plus Polyoxidonium (group 3) (Figure 3). Difference between group 2 and group 3 was significant ($p < 0.01$) that demonstrates immunopotentiation of antibody responses by Polyoxidonium.

Table 3. Correlation between dynamics of citokine's changing (Pearson's correlation matrix).

	IL–1	IL–2	IL–4	IL–5	IL–6	IL–7	IL–8	IL–10	IL–12	THF–α	IFN–γ
IL–1	1.000	**0.705**	0.354	0.189	**0.756**	**–0.654**	0.528	**–0.810**	**–0.602**	0.311	0.469
IL–2		1.000	**0.890**	–0.244	**0.913**	–0.507	0.143	–0.565	**–0.768**	0.207	**0.640**
IL–4			1.000	–0.223	**0.821**	–0.354	0.019	–0.337	–0.497	0.325	**0.747**
IL–5				1.000	0.145	–0.477	0.503	–0.484	**0.660**	**0.829**	0.446
IL–6					1.000	**–0.777**	0.220	**–0.808**	–0.512	0.513	**0.797**
IL–7						1.000	–0.024	**0.970**	0.196	–0.499	–0.507
IL–8							1.000	–0.223	0.094	0.599	0.513
IL–10								1.000	0.284	–0.516	–0.538
IL–12									1.000	0.423	–0.016
THF–α										1.000	**0.862**
IFN–γ											1.000

Note: Significant correlations ($p < 0.05$) are in bold.

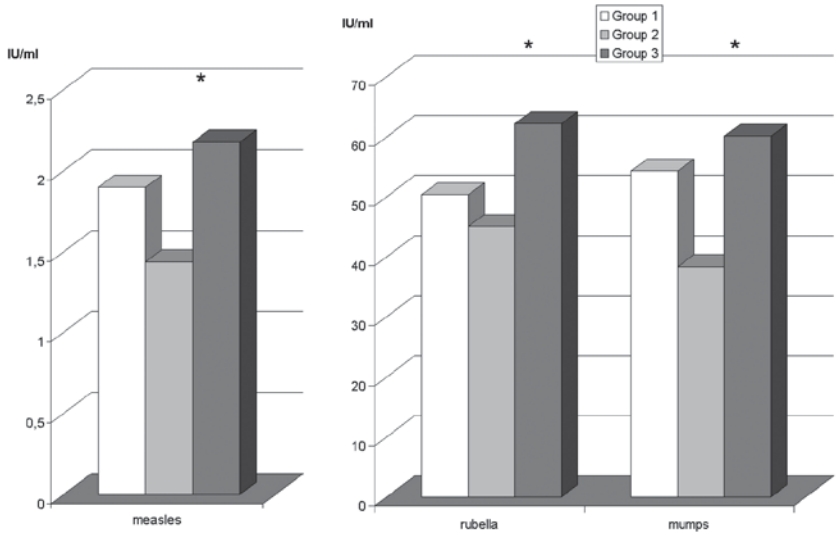

Figure 3. Influence of a Polyoxidonium injection on the antibody response in children vaccinated with Priorix. Measles IgG-antibody is presented in International Units per ml, rubella and mumps antibodies are expressed in Human's Units per ml. The serum samples were collected 4 weeks after vaccination. *Differences between Group 2 and Group 3 were significant ($p < 0.01$ using tow-sided t-test). See the legend to Figure 1.

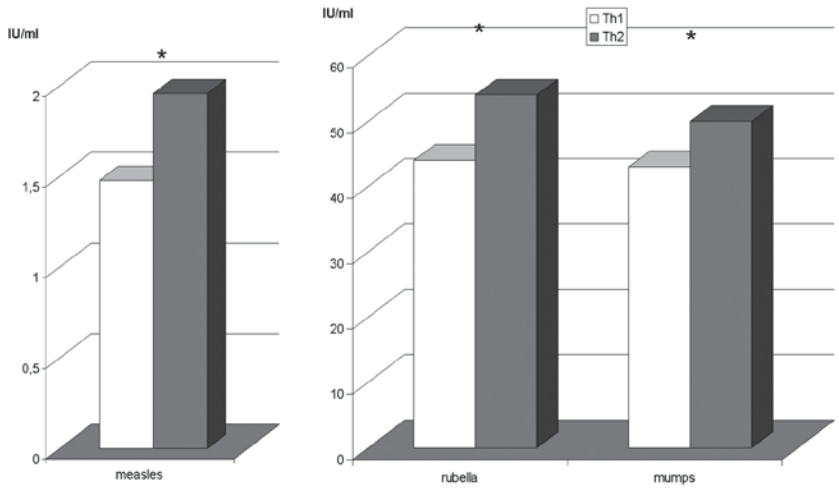

Figure 4. Influence of Th1 or Th2 type of immune response on IgG-antibody level in children vaccinated with Priorix. See the legend to Figure 3. *$p < 0.05$ using tow-sided t-test.

Antibody response against each component of the trivalent vaccine was significantly higher ($p < 0.05$) in Th2 responding children than those who demonstrated Th1 type immune response (Figure 4). The level of serum IgE relatively decreased in 48% children on seventh day after vaccination, and increased in 52%. In a month IgE level returned back. A significant positive correlation was observed between IgE level increasing and Th2 type of immune response ($\chi^2 = 15$; $p < 0.001$). Despite the skewing of immune response from Th1 to Th2 in children from group 3, we did not observe a prevalence of IgE level increasing in this group.

Discussion

In contrast to immunologic abnormalities with measles patients, neither lymphopenia, nor changing in lymphocyte subpopulation occurred with measles vaccine recipients (Hussey *et al.*, 1996, Munyer *et al.*, 1975). On the other hand, lymphopenia and decreased $CD4^+$ and $CD8^+$ counts have been described for vaccinated subjects (Ovsyannikova *et al.*, 2003a; Rager-Zisman *et al.*, 2003). It may be explained by the usage of different virus strains (Edmonston B, Edmonston-Zagreb, Schwarz) or predominant genetic differences between population (Rager-Zisman *et al.*, 2004). We did not observe any significant difference in the characteristics of lymphocyte subsets one month after vaccination. We have also found no effect of vaccination on lymphocyte numbers and $CD4^+$ and $CD8^+$ T cell content in children on the seventh day in total. We have shown, however, that children of the same population and vaccinated with the same vaccine demonstrated different types of reaction on vaccination. In some children on the seventh day after vaccination,

numbers of lymphocytes and each subset were relatively decreased compared with initial levels. In the samples of the other children the numbers of lymphocyte and T cell subsets tended to recover to the normal ranges seven days later after the immunization (see Table 1). We believe that differences observed on the seventh day are associated with the macro-organism's status. Those children, who demonstrated decreased T cell counts on day seven, had never been ill and their indices of immunity corresponded to the survival rate. In contrast, those who demonstrated increasing numbers of lymphocytes and T cell subpopulation on the seventh day had some abnormalities of their immune system. We named this group "immunocompromised children". They had no real problems with their immune system such as HIV infection or leukaemia. However these 1- to 2-year-old children had one or more episodes of respiratory infection and their T cell subset counts had been decreased. The differences in the characteristics of cellular immunity between group 1 and groups 2 and 3 were significant ($p < 0.01$). It was shown that Polyoxidonium could smooth out the immune reaction on vaccination, and "immunocompromised" children demonstrated response like healthy children.

Serum cytokine concentration corresponds to the immune process in the whole organism. A number of studies have reported a preferential activation of IL-4 producing Th2 cells by measles vaccine (Ward and Griffin, 1993). On the other hand, it has been demonstrated that a predominant Th1 cytokine pattern occurred after measles vaccination (Ovsyannikova *et al.*, 2003b). The similar results were obtained in rubella vaccinated girls (Pukhalsky *et al.*, 2003). Moreover, it has been shown that measles virus vaccination may maintain either Th1 or Th2 cytokine production (Dhiman *et al.*, 2005). It has also been found that measles virus haemagglutinin primes for a type 2 cytokine response, while the fusion protein primes for a type 1 response (Polack *et al.*, 2003). Involving the second derivative from cytokine concentration function allowed us to avoid the breaking up persistent changing in Th1/Th2 balance into 'early' and 'late' events (Moss *et al.*, 2002; Tetteeh *et al.*, 2003).

Dynamic of cytokine concentration has shown that about 50% vaccinated children developed Th1 type response while the others demonstrated Th2 type. Th2 type of immune response is accompanied by higher level of specific antibodies and transitory elevation of IgE. On the contrary, Th1 immune response demonstrates lower, but protective, level of specific antibodies.

We have found no change in IL-5 (a cytokine, associated with allergy) and IL-12 (the cytokine-inductor of Th1 type response). It is known that measles virus can down-regulate IL-12 and IL-5 production (Carsillo *et al.*, 2009; Gans *et al.*, 2008; Polack *et al.*, 2002). We think that the lack of change in pro-inflammatory cytokines IL-1, IL-6, and IL-8 is a very positive reaction to vaccination.

Our findings indicate that the healthy children need no fortification of their immune responses on Priorix vaccination, because they can produce a high level of specific antibodies. However, children with previous exposure to harmful factors affecting normal T cell content (viral and other diseases) may demonstrate a lower level of specific IgG antibodies and use of Polyoxidonium in this group of children in concert with Priorix vaccine can improve the induction of immunity against measles, rubella, and mumps. On the other hand, increase in TNF- level and skewing the dominant immune responses from Th1 to Th2 type could not be appreciated as positive effect of Polyoxidonium. Indeed, it has been shown that Th1 dependent cell-mediated immunity is essential for recovery from virus infection, and children with isolated agammaglobulinemia recover from measles and develop lifelong immunity to this infection (Good and Zak, 1956). Additional epidemiological and immunological studies are needed to determine whether the Polyoxidonium contributes to a further decrease in incidence of infections after vaccination and/or affects recovery after the infection and if the specific cell-mediated immune responses are impaired in those children.

Declaration of interest

Authors declare no conflict of interest.

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