

**BACKGROUND and aim:** Immunization with live virus vaccines may cause an immunosuppression with lymphopaenia, impaired cytokine production and defective lymphocyte response to mitogenes. These abnormalities were described in subjects vaccinated against measles. This study was performed to analyse the host immune response related to immunosuppression in subjects vaccinated with live attenuated rubella vaccine.

**Methods:** Eighteen schoolgirls, aged 11–13 years, were vaccinated with live attenuated rubella vaccine *Rudivax*<sup>®</sup>. Before immunization, and 7 and 30 days after, peripheral blood was collected. Cellular fractions were subjected to flow cytometric analysis, and the lymphocyte response to phytohaemagglutinin was investigated. Plasma samples were analysed for cytokines (interleukin (IL)-4, IL-10, tumour necrosis factor- $\alpha$ , and interferon- $\gamma$ ) by enzyme-linked immunosorbent assay techniques.

**Results:** On day 7 after vaccination, the number of each lymphocyte subset was decreased; however, only for CD3 and CD4 lymphocytes has a significant reduction been shown. On the contrary, tumour necrosis factor- $\alpha$  and IL-10 levels markedly increased and amounted to its maximum on day 30. Simultaneously, a significant reduction in plasma interferon- $\gamma$  and a profound decrease of the lymphocyte response to phytohaemagglutinin were shown. The changes were accompanied with marked elevation of plasma IL-4.

**Conclusions:** Our data indicate that the vaccination with live attenuated rubella vaccine results in moderate but sustained immune disturbance. The signs of immunosuppression, including defective lymphocyte response to mitogene and impaired cytokine production, may persist for at least 1 month after vaccination.

**Key words:** IFN $\gamma$ , IL-4, IL-10, Immunosuppression, Lymphocyte response to phytohaemagglutinin, Lymphocyte subsets, Rubella vaccine, TNF- $\alpha$ , Vaccination

## Cytokine profile after rubella vaccine inoculation: evidence of the immunosuppressive effect of vaccination

Alexander L. Pukhalsky<sup>1,CA</sup>, Galina V. Shmarina<sup>1</sup>,  
Maria S. Bliacher<sup>2</sup>, Irina M. Fedorova<sup>2</sup>,  
Anna P. Topygina<sup>2</sup>, Julia J. Fisenko<sup>2</sup> and  
Vladimir A. Alioshkin<sup>2</sup>

<sup>1</sup>Research Centre for Medical Genetics, 1  
Moskvorechie Street, Moscow, 115478, Russia;

<sup>2</sup>Moscow G.N. Gabrichevsky Institute of  
Epidemiology and Microbiology, Moscow, Russia

<sup>CA</sup>Corresponding author

Fax: +7 95 324 0702

E-mail: osugariver@medgen.ru, osugariver@mtu-net.ru

## Introduction

Natural rubella virus infection is characterized by fever, sore throat, lymphadenopathy, and skin rash, which usually resolve quickly.<sup>1</sup> It has been estimated that about one-half of all rubella virus infections are clinically inapparent<sup>2</sup> and would only be detected serologically.<sup>3,4</sup> Since maternal rubella infection during the first trimester of pregnancy may result in spontaneous abortion or in foetal infection, leading to serious birth defects,<sup>5</sup> it has become routine practice to offer immunization to all seronegative women of childbearing age as well as to infants. When such vaccination programmes have been undertaken, the incidence of reported rubella and

congenital rubella syndrome has declined.<sup>4</sup> Despite the fact that the epidemiology of rubella has changed significantly in the past decade, it is generally agreed that the current strategy of vaccination must be enhanced.<sup>6</sup> It is well known that immunization with live virus vaccines may cause slight but not negligible clinical manifestations such as fever, skin rash and immunosuppression with lymphopaenia, impaired cytokine production and defective lymphocyte response to mitogenes.<sup>7–9</sup> These abnormalities have been described in detail in subjects vaccinated against measles.

This study aimed to analyse the host immune response related to immunosuppression in subjects vaccinated with live attenuated rubella vaccine.

## Materials and methods

### Subject assessment

The study group included 18 schoolgirls, aged 11–13 years, with a negative history of rubella. All subjects had routine physical examination and rubella serotesting. None of the participants were seropositive. Girls were vaccinated with live attenuated rubella vaccine Rudivax<sup>®</sup> (Aventis Pasteur, Lyon, France). No vaccine-related adverse effects were noted. Blood samples were collected before vaccination, and then again 1 week and 1 month later. All study subjects showed protective titres of immunoglobulin G, defined as >1:400, after immunization. The study was approved by the Ethics Committee of the Moscow G.N. Gabrichevsky Institute of Epidemiology and Microbiology.

### Blood collection

Blood was collected in tubes with heparin (25 IU/ml) by venopuncture.

### Flow cytometry

Mononuclear cells were isolated from heparinized peripheral blood by Ficoll-Verographin density gradient centrifugation. Cellular fractions were subjected to flow cytometric analysis (FACSCalibur, Becton-Dickinson, Franklin Lakes, NJ, USA) for counting absolute numbers of cell populations. The samples were treated directly with five monoclonal antibodies labelled with fluorescein isothiocyanate (CD3/CD4/CD8/CD16/CD19; SORBENT, Moscow, Russia) for analysing lymphocyte subsets.

### Cytokine assay

Plasma samples were harvested and analysed for cytokines by enzyme-linked immunosorbent assay techniques with commercially available kits (Cytimmune Science Inc., College Park, MD, USA). The enzyme-linked immunosorbent assay kits for interleukin (IL)-4, tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and IL-10 were designed to measure the 'total' (bound and unbound) amount of the cytokines. The kit for interferon- $\gamma$  (IFN $\gamma$ ) was designed to measure the 'free' forms of the cytokine.

### Peripheral blood lymphocyte response to phytohaemagglutinin

Mononuclear cells were isolated from heparinized peripheral blood by Ficoll-Verographin density gradient centrifugation. The cells were washed twice and re-suspended in RPMI-1640 medium supplemented with 10% heat-inactivated donor horse serum,

$2 \times 10^{-3}$  M of HEPES, 2 mM of L-glutamine,  $2.8 \times 10^{-6}$  M of 2-mercaptoethanol, and 20  $\mu$ g/ml of gentamycin. The cells were cultivated in flat-bottomed 96-well plates, and contained  $5 \times 10^4$  cells in each well. The final concentration of phytohaemagglutinin (PHA) was 5  $\mu$ g/ml. The cells were incubated for 72 h at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. Four hours before the end of cultivation, each well was pulsed with 40 kBq of [<sup>3</sup>H]-thymidine. The cells were harvested with a cell harvester and counted on a liquid scintillation counter. Triplicate wells were assayed and the counts per minute were averaged.

### Statistical analysis

Statistical analysis was performed using non-parametric Wilcoxon tests.

## Results

### Lymphocyte subsets

Absolute numbers of total lymphocytes and lymphocyte subsets were determined for peripheral blood of vaccine recipients as described in Materials and methods. Results obtained before and after vaccination are presented in Table 1. It was noted that on day 7 after vaccination, average numbers of total lymphocytes and each subsets were relatively decreased compared with initial levels. However, only for CD3 and CD4 lymphocyte subsets has a significant reduction been revealed. The numbers of total lymphocyte and T-cell subsets tended to recover to the normal ranges within 1 month, whereas the number of B cells remained at a low level.

### Plasma cytokines

As can be seen in Fig. 1, a transient increase of IFN $\gamma$  levels on day 7 after rubella vaccine inoculation has been observed ( $p = 0.028$ ). One month later, this parameter did reduce dramatically, down to 0 in several subjects ( $p = 0.009$ ). Fig. 2 illustrates the individual variations in IL-4 levels measured before vaccination and then again 7 and 30 days after. Significant elevation of the cytokine levels at day 30 after vaccine inoculation was found.

We further investigated the plasma concentration of TNF- $\alpha$  and IL-10, a pair of cytokines that play opposite roles in the immune response. After rubella vaccination, a marked increase in both IL-10 and TNF- $\alpha$  concentrations has been observed (Fig. 3). The relative concentrations of plasma TNF- $\alpha$  and IL-10 were also calculated. Before vaccination, most children (13 of 18) exhibited a relatively high cytokine ratio (median value, 1.56). On day 7 after

**Table 1.** Effect of rubella vaccination on numbers of lymphocytes in the peripheral blood (absolute lymphocyte count/ $\mu$ l)

	Before inoculation	7 days after inoculation	30 days after inoculation
Total lymphocytes	2046 $\pm$ 161	1617 $\pm$ 159	1868 $\pm$ 109
CD3 <sup>+</sup>	1214 $\pm$ 110	936 $\pm$ 85*	1118 $\pm$ 60
CD4 <sup>+</sup>	747 $\pm$ 68	550 $\pm$ 59**	659 $\pm$ 48
CD8 <sup>+</sup>	481 $\pm$ 51	376 $\pm$ 36	444 $\pm$ 40
CD4/CD8	1.61 $\pm$ 0.11	1.55 $\pm$ 0.15	1.58 $\pm$ 0.14
CD16 <sup>+</sup>	340 $\pm$ 34	257 $\pm$ 34	286 $\pm$ 28
CD19 <sup>+</sup>	448 $\pm$ 61	392 $\pm$ 64	383 $\pm$ 53

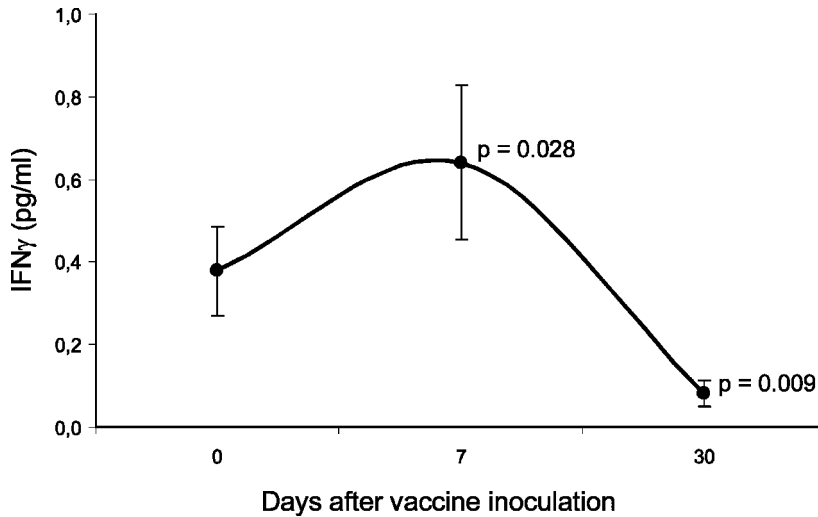
Data presented as the mean  $\pm$  standard error of the mean.

\* $p = 0.05$ , \*\* $p = 0.038$  using the paired Wilcoxon test.

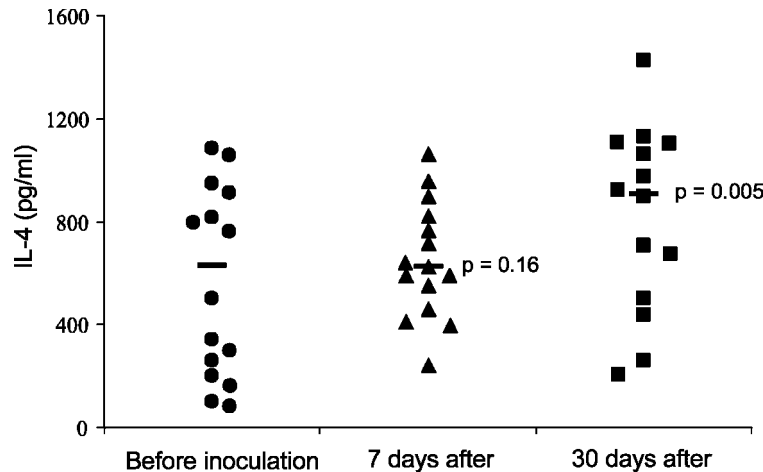
immunization, a rise in IL-10 passed ahead of TNF- $\alpha$  expansion. In this context, a significant decrease in the TNF- $\alpha$ /IL-10 ratio was observed (median value, 0.65;  $p = 0.05$ ). On day 30 after rubella vaccination, IL-10 still predominated over TNF- $\alpha$  and the median value of the cytokine ratio did not exceed 0.52 ( $p = 0.02$ ).

**Proliferative response to PHA**

The influence of rubella vaccine inoculation upon PHA-induced peripheral blood lymphocyte response is summarized in Table 2. There were no significant differences in levels of T-lymphocyte proliferation before vaccination and 7 days later. At the same time,



**FIG. 1.** IFN $\gamma$  dynamics after live attenuated rubella vaccine inoculation. Results are expressed as the mean  $\pm$  standard error of the mean. Data reported were compared by the two-sample Wilcoxon test.



**FIG. 2.** Individual variations in IL-4 levels after rubella vaccine inoculation. The median values are designated with horizontal lines. Each dot represents one subject. The data were analysed using the two-sample Wilcoxon test.

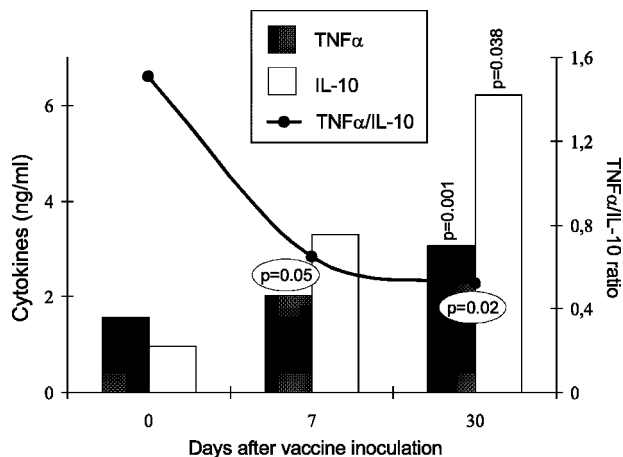


FIG. 3. Changes of TNF- $\alpha$  and IL-10 levels in plasma after rubella vaccine inoculation. The plasma concentrations of both cytokines rose at day 30 after vaccine inoculation, whereas the TNF- $\alpha$ /IL-10 ratio significantly decreased since day 7. Results are presented as median values. Data reported were compared by the two-sample Wilcoxon test.

**Table 2.** Effect of rubella vaccine inoculation on PHA-induced peripheral blood lymphocyte response

	Proliferative response (count/min)	<i>p</i>
Before inoculation	26630 $\pm$ 4213	
7 days after	34650 $\pm$ 4730	0.45
30 days after	13848 $\pm$ 2088	0.01

Data presented as the mean  $\pm$  standard error of the mean. Data compared by the paired Wilcoxon test.

a profound decrease of T-cell proliferation value on day 30 following vaccination was observed.

## Discussion

The immune response to virus infections and attenuated virus vaccines seems to depend on various factors including genetics, age and sex. Thus, measles patients of 1–2 years of age are least severe in clinical symptoms and lymphopaenia, whereas young infants before their first birthday as well as adolescents and adults will progress to severe conditions.<sup>10</sup> Studies from Guinea-Bissau and South Africa have observed increased mortality due to measles in girls,<sup>11,12</sup> and girls are more likely to develop fever and rash after routine measles–mumps–rubella immunization.<sup>13</sup> Similarly to measles vaccine recipients,<sup>14</sup> most schoolgirls enrolled in our study demonstrated moderate lymphopenia after rubella vaccine inoculation. In particular, on day 7 after vaccination the number of each lymphocyte subset was decreased compared with the initial level, and for CD3 and CD4 lymphocyte subsets a significant reduction has been revealed. The mechanism by which the numbers of lymphocytes are decreased during viral diseases and after vaccine inoculation is unclear. Some authors

postulate that transient lymphopaenia arises from virus-induced death of T cells.<sup>10</sup> Another potential mechanism for change in the number of circulating lymphocytes may be associated with alterations in lymphocyte trafficking, which related to increased production of IFN $\gamma$ .<sup>15,16</sup> It is significant that in our study on day 7 after rubella vaccination, transient elevation in plasma IFN $\gamma$  has been observed (see Fig. 1).

In contrast to the rubella patients demonstrating a transient increase in IL-10 level,<sup>17</sup> a sustained elevation of the cytokine concentration in rubella vaccine recipients has been found. The IL-10 levels increased as early as day 7 and amounted to a maximum on day 30. Simultaneously, a significant reduction in plasma IFN $\gamma$  and a profound decrease of peripheral blood lymphocyte response to PHA have been shown. These changes were accompanied with marked elevation of IL-4. It may be proposed that the observed cytokine ‘shift’ from type 1 cytokines early after vaccine inoculation to type 2 cytokines 1 month after vaccination is associated with activation of central mechanisms of immunosuppression. This assumption is indirectly confirmed by changes in TNF- $\alpha$ /IL-10 ratios after rubella vaccine inoculation. Thus, before vaccination, most girls (13 of 18) exhibited relatively high cytokine ratios (TNF- $\alpha$ /IL-10 > 1), demonstrating predominance of pro-inflammatory TNF- $\alpha$  over anti-inflammatory IL-10. Rubella vaccination and associated stress were bound to result in catecholamine and corticosteroid releasing that promoted IL-4 and IL-10 synthesis.<sup>18</sup> Besides, auxiliary sources of anti-inflammatory IL-10 (such as liver, spleen and brain as well) might be activated.<sup>19,20</sup> Thereafter, on day 7 after immunization, a rise in IL-10 passed ahead of TNF- $\alpha$  expansion. In this context, a significant decrease of TNF- $\alpha$ /IL-10 ratios has been observed. On day 30 after rubella vaccination IL-10 still predominated over TNF- $\alpha$ , suggesting moderate but sustained immune disturbance.

In conclusion, live attenuated rubella vaccine inoculation may cause sustained immunosuppression including defective lymphocyte response to mitogene and impaired cytokine production. The signs of immunosuppression may persist for at least 1 month after vaccination.

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