MURINE CYTOKINE PATTERNS FOLLOWING RUBELLA VACCINATION

Parvaneh Farzaneh,* Massoumeh Ebtekar,* Z.M. Hassan,* Sima Rafati**

*From the *Department of Immunology, Tarbiat Modarres University, Tehran, Iran.*
***Department of Immunology, Pasteur Institute of Iran, Tehran, Iran.*

ABSTRACT

Although thorough studies on the immune reponse to *rubella* have been performed, less attention has been given to the cellular mechanism and mediators that shape the process. Specifically, information concerning the nature of cytokine patterns involved in the immune response to *Rubella* vaccination is not avaliable. This study deals with cytokine production patterns of spleen cells from Balb/c mice following vaccination with the Takahashi strain of Rubella vaccine. Mice were injected intraperitonealy with Rubella virus and PBS and 7, 10 or 14 days later, spleen cells were separated and cultured with varying doses of virus, con A or only the medium. ELISA assays were performed on supernatants for measurement of IL-4, INF-γ and IL-5. LTT (Lymphocyte Transformation Test) was also performed. The data indicate variation in cytokine patterns during the time periods after vaccination. On day 7 a type 1 pattern was observed. The LTT response was also indicative of CMI (Cell Mediated Immunity) response on the 7th and 14th days while a transient suppression on day 10 was observed. These results indicate a time dependent cytokine response with variation ultimately leading to a dominant type 1 (T1) cytokine response.

Keywords: Rubella, cytokine production pattern, Takahashi vaccine, Cell mediated immunity.

INTRODUCTION

Rubella has initially been described as a mild viral disease with symptoms like fever, skin blisters, lymphadenopathy and arthritis.[8] However today we know that other than causing encephalitis in very rare instances,[13] in cases where viral persistence occurs, a string of autoimmune disease like insulin dependent diabetes (IDDM), arthritis and disturbances in thyroid and pituitary functions may ensue.[9,13] On the other hand, if a non-immune mother contacts the disease during the first term of her pregnancy, her newborn may develop the Congenital *Rubella* Syndrome (CRS). The occurresnce of this syndrome has been reported even in newborns with vaccinated mothers.[8,5,13]

This study which focuses on the development of the immune response during a specific time period following infection with *Rubella* or subsequent to vaccination can enable us to undertand the underlying pathological mechanisms involved in the autoimmune complications, CRS and encephalitis. Also studies elucidating the molecular mechanisms regulating the immune response, could benefit in understanding similar con-

Corresponding Author: Massoumeh Ebtekar, Fax: 0098 021 8963110, E-mail: yaraei_r@modares.ac.ir or, ryaraee@yahoo.com.

ditions occurring in other viral diseases and the development of new, more safe and effective vaccines.

Cytokines are known for their ability to direct and modulate the immune response.[12] Two distinct patterns of cytokines have been defined; type one cytokines,[11] which induce a CMI directed response and type two cytokines, which favor a humoral response.[1] The types and levels of cytokines produced after vaccination can provide an insight into the cytokine patterns involved and the nature of the ensuing protective response. This model could also provide information on the direction of immune response following infection with Rubella. This would broaden our understanding of the underlying mechanisms and of the important complications observed subsequent to this common viral disease.

MATERIALS AND METHODS

Animals: Female, 6-8 weeks Balb/c mice were obtained from the Razi Institute, Karaj, Iran. They were fed standard mouse chow and water and libitum.

Antibodies: Monoclonal antibodies for ELISA were purchased from Pharmingen, USA.

Antigens: Concanavalin A was obtained from Sigma; the Takahashi *Rubella* vaccine was purchased from the Razi Institute, Karaj, Iran.

Immunization: After optimization of injecting dose, mice were injected intraperitoneally with 200 IND50 (infecting dose 50%) Takahashi *Rubella* vaccine, control groups received PBS alone. Each group consists of five mice.

Single cell suspensions: Mice were sacrificed 7, 10 or 14 days after vaccination, single cell suspensions were prepared from their spleens and cultured in RPMI 1640, supplemented with 100 U/mL Penicillin and 100μg/mL Streptomycin and 2 mM L-Glutamine, all purchased Sigma.
Inactivated fetal calf serum (FCS) 10% from Sigma was also employed.
96-well flat-buttomed microtiter plates (Nune, Denmark) were used for the *in vitro* stimulation of cells.

Cytokine production and assay: 4×10^5 spleen cells were incubated with con-A, 50 IND50, 100 IND50 or 200 IND50 Takahashi virus, Kanamycine (as supplemented in the vaccine) for 48 hrs (optimization of time was done by comparing of 24, 48, 72, 96, 132 hrs culture results) in 5% CO_2 at 37°C. Supernatants were assayed for IL-4, IL-5 and IFN-γ in 96-well plates using sandwich ELISA technique. The results are presented as optical density.

Lymphocyte transformation test: LTT was done as usuall method (2). The results are presented as stimulation index (SI= The ratio of the mean cpm or count per minute of experimental cultures to the mean cpm of control cultures).

Statistics:
In this study one-way analysis of variation (ANOVA) and the Kruskal-Wallis nonparametric test were employed using SPSS software ($p<0.05$).

RESULTS

The initial stage of this work consisted of efforts aimed at determining the cytokine pattern subsequent to *Rubella* vaccination in an animal model in Balb/c mice.

IL-4 production during infection with *Rubella* virus

In order to evaluate the IL-4 production during *rubella* infection in mice, three doses of virus inoculated according to the protocol in Table I.

Results indicated a significant ($p<0.05$) incrase in IL-4 in the positive control conA on day 7. No significant differences were noticed in *rubella* vaccinated groups.

IL-5 production during infection with rubella virus

Levels of IL-5 production during the rubella infection were assayed according to the protocol in Table II. Results indicate no significant ($p<0.05$) differences among all groups compared to controls.

IFN-γ production during infection with *rubella* virus

In order to assess the level of IFN production, assays were performed based on the protocol in Table III. The results in Table III indicate that IFN showed a significant ($p<0.05$) increase on the day 7 in rubella vaccinated groups in 100 and 200 IND50 virus treatment. It seems that the results of 100 and 200 IND50 treatments did not show signficant differences but they showed significant differences with 50 IND50 treatment. Con A stimulated cells also showed a significant ($p<0.05$) increase during the 7th and 14th days.

Lymphocyte transformation response to rubella vaccine

In order to assess the percent of stimulation index for *rubella* virus and conA the protocol in Table IV was applied. The results in Table IV indicate a significant ($p<0.05$) increase among rubella vaccinated groups after 7 days. Con A stimulated cells also showed a sig-

Table I. IL-4 production by spleen cells after stimulation with Takahashi *Rubella* Vaccine

Time of Vaccination	Doses of Vaccine (IND50[c] virus) 50	100	200	Kanamycine	Con A[b]	PBS[a]
Group 1						
7 days	0.555±0.03	0.533±0.03	0.544±0.03	0.503±0.04	1.203±0.04	0.541±0.02
Control	0.498±0.04	0.504±0.03	0.514±0.05	0.449±0.02	0.891±0.1	0.495±0.03
Group 2						
10 days	0.518±0.02	0.508±0.01	0.529±0.03	0.519±0.01	0.795±0.02	0.41±0.05
Control	0.525±0.07	0.525±0.05	0.549±0.02	0.516±0.03	0.970±0.01	0.493±0.05
Group 3						
14 days	0.461±0.01	0.450±0.005	0.470±0.05	0.492±0.03	1.048±0.4	0.524±0.05
Control	0.471±0.07	0.483±0.02	0.479±0.07	0.490±0.005	0.811±0.01	0.519±0.03

*Data presented in optical density with SD ($p<0.05$)
[a]Phosphate Buffered Saline
[b]concanavalin A
[c]infecting Dose 50

Table II. IL-5 production by spleen cells after stimulation with Takahashi *Rubella* Vaccine

Time of Vaccination	Doses of Vaccine (IND50[c] virus) 50	100	200	Kanamycine	Con A[b]	PBS[a]
Group 1						
7 days	0.500±0.03	0.485±0.03	0.496±0.02	0.536±0.03	0.561±0.01	0.535±0.01
Control	0.539±0.03	0.523±0.05	0.522±0.03	0.544±0.02	0.529±0.02	0.519±0.02
Group 2						
10 days	0.569±0.04	0.547±0.01	0.538±0.06	0.548±0.02	0.564±0.06	0.582±0.09
Control	0.520±0.02	0.519±0.01	0.544±0.02	0.531±0.04	0.516±0.01	0.530±0.03
Group 3						
14 days	0.483±0.04	0.455±0.05	0.473±0.03	0.539±0.03	1.599±0.1	0.559±0.07
Control	0.557±0.07	0.526±0.04	0.500±0.003	0.557±0.003	0.542±0.03	0.507±0.05

*Data presented in optical density with SD ($p<0.05$)
[a]Phosphate Buffered Saline
[b]Comcanavalin A
[c]Interfering Dose 50

nificant ($p<0.05$) increase on the 7th and 14th days.

DISCUSSION

The cytokine patterns emerging following antigen vaccination or infection with certain pathogens can determine the outcome of the immune response. This pattern may also exert a profound influence on the resolution of viral infections and clearance.[10] These

Table III. IFN-γ production by spleen cells after stimulation with Takahashi *Rubella* Vaccine

Time of Vaccination	Doses of Vaccine (IND50[c] virus) 50	100	200	Kanamycine	Con A[b]	PBS[a]
Group 1						
7 days	0.206±0.01	0.392±0.01	0.413±0.02	0.210±0.004	0.430±0.03	0.215±0.01
Control	0.204±0.01	0.208±0.04	0.205±0.007	0.207±0.01	0.265±0.05	0.214±0.006
Group 2						
10 days	0.286±0.05	0.230±0.007	0.245±0.07	0.300±0.07	0.439±0.02	0.267±0.04
Control	0.204±0.01	0.205±0.009	0.205±0.01	0.207±0.009	0.367±0.01	0.218±0.003
Group 3						
14 days	0.242±0.01	0.240±0.009	0.261±0.02	0.234±0.01	1.496±0.09	0.237±0.006
Control	0.204±0.01	0.211±0.004	0.204±0.05	0.207±0.009	0.260±0.03	0.209±0.007

*Data presented in optical density with SD ($p<0.05$)
[a]Phosphate Buffered Saline
[b]Concanavalin A
[c]Interfering Dose 50

Table IV. Lymphocyte Transformation Test after stimulation with Takahashi *Rubella* Vaccine

Time of Vaccination	Doses of Vaccine (IND50[c] virus) 50	100	200	Kanamycine	Con A[b]	PBS[a]
Group 1						
7 days	0.94±0.02	2.4±0.02	2.1±0.5	1.06±0.2	2.53±0.2	1
Control	0.8±0.08	0.98±0.2	0.83±0.08	0.98±0.1	5.8±0.9	1
Group 2						
10 days	_____	0.65±0.07	_____	0.98±0.07	0.88±0.07	1
Control	_____	0.86±0.1	_____	1.1±0.1	0.95±0.2	1
Group 3						
14 days	1.4±0.1	1.7±0.1	1.8±0.1	1.2±0.2	7.2±2	1
Control	0.80±0.2	1.1±0.2	0.83±0.3	0.86±0.1	6.1±0.8	1

*Data presented in optical density with SD ($p<0.05$)
[a]Phosphate Buffered Saline
[b]Concanavalin A
[c]Interfering Dose 50

cytokines may also be responsible for the subsequent complications. Therefore a precise understanding of ensuing cytokine patterns and activation mechanism in each response is essential.

Previous studies showed that fourteen days after MMR vaccination of the breastfed children, increased

production of interferon-gamma ($p<0.02$) and incrased percentages of CD56+ ($p<0.022$) and CD8+ cells ($p<0.004$) could be seen. These findings are consistent with a Th1 type response by breastfed children.[7] Another study showed that after MMR vaccination interferon-gamma was the principal cytokine produced after primary measles immunization, suggesting that primary measles immunization induced predominantly a TH1 type response.[6] These studies didn't follow the cytokine pattern outcome of *Rubella* vaccine specifically.

Our work included a dose response study on the effect of varying doses of the virus. Both cytokine and LTT tests indicated a significant response at 100 IND50 and 200 IND50 virus. Lower doses (50 IND50) could not induce a significant cytokine and LTT response. No significant difference in cytokine levels or LTT was observed between the 100 and 200 doses indicating that responses were not dose dependent at this stage.

This study indicates that a type 1(T1) polarized response characterized by significant levels on IFN-gamma and undetectable levels of IL-4 and IL-5 follows vaccination with Rubella Takahashi strain. This pattern is detectable 7 days later while on days 10 and 14 cytokine levels are not significant. Since con A is a mitogen with polyclonal activation, cytokine production and LTT responses to con A can be expected from both committed as well as uncommited cells. The results show a significant suppression in LTT response 10 days after vaccination which can be due to secretion of suppressor factors.

The predominance of IFN-gamma and cellular responses we encountered upon restimulation can account for the protection provided by this vaccine. In cases of infection while the Type 1 response would be favorable, the polarization of the immune response could result in unwanted effects. Interferon gamma is a proinflammatory cytokine known to activate T and NK cells, macrophages, increase MHC class I and II expression and enhance cellular responses.[4] Overproduction of T1 cytokines is implicated in the etiology of many autoimmune diseases and the role of IFN-gamma is well documented.[3] Considering the variety of complications (autoimmune diseases and arthritis) attributed to rubella infection, our data indicate that the role for IFN-gamma in this process must not be overlooked.

Further studies on other cytokines involved in the response, their kinetics and patterns is necessary to shed light on the immune response following *Rubella* infection. These studies may also provide insight into the underlying mechanisms of complications associated with *Rubella* and thus the necessary preventive and therapeutic measures to be taken as well.

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