Gamma interferon levels among Bangladeshi children after measles vaccination

Sultana S, Tabassum S, Nessa A, Jahan M

Department of Virology, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka.
Email: sharmins34@yahoo.com

Abstract

Gamma interferon (IFN-γ) plays an important role in the immune response to live measles virus vaccination. To study the immune response to measles vaccination, IFN-γ level was estimated in 30 children. Of these, 24 children vaccinated with a single dose of measles vaccine at nine months of age and 06 children vaccinated with a second dose during the Measles Catch-up Immunization campaign. Measles vaccine strain was cultured in Vero cell line and the Tissue Culture Infective Dose $(TCID)_{50}$ was used as standard live virus. Peripheral blood Mononuclear cells (PBMCs) was separated by Ficoll-Hypaque density gradient centrifugation and stimulated with measles virus antigens and mitogens (lectin), cultured in CO_2 and IFN-γ level was measured from culture supernatant by ELISA. On stimulation with measles antigen and lectin respectively, IFN-γ level was highest (105 pg/ml and 226.54 pg/ml) in the 109-120 months age group while it was lowest (12.97 ± 8.16 pg/ml and 13.16 ± 8.0 pg/ml) in the 61-72 months age group. No significant difference was observed in IFN-γ level after stimulation with either measles antigen or lectin among well-nourished (p<0.8) and mal-nourished (p<0.7) children suggesting that nutritional status did not have any effect on IFN-γ level. However, IFN-γ level was higher in children who received two dose of measles vaccine than those who received a single dose (p<0.001).

Introduction

Measles is a major cause of childhood morbidity and mortality especially among young children from developing countries like Bangladesh. In the absence of proper immunization programmes, it affects nearly every person in a given population by adolescence.[1] Measles vaccine induces both humoral immunity and cell mediated immune (CMI) responses. Humoral immunity to measles vaccine has been extensively studied, and it is believed that both primary vaccine failure (complete lack of antibody after immunization) and secondary vaccine failure (waning or insufficient antibody after immunization) may be responsible for the variable efficacy of measles vaccine.[2-4] It is speculated that measles vaccine failure may result from the induction of a polarized cytokine profile, which induces an immune response that may not be sufficiently protective.[5] Although humoral immunity plays a pivotal role in protection against measles, several reports suggest that CMI is essential for recovery from measles and may provide long-term immunity.[6-9] This is best illustrated in children with isolated agammaglobulinemia who recover from measles and develop lifelong immunity.[10] In contrast, individuals with T-cell deficiencies, such as, leukemia and human immunodeficiency virus (HIV) infection experience a progressive illness with 50-100% mortality.[11-12] These findings clearly suggest that the cellular immune response plays a central role in immunity to measles infection and in protection against reinfection.

Despite the importance of cellular responses in measles, relatively little is known about the cellular immune response induced by measles vaccination. Subpopulation within the classical CD4+T lymphocyte subsets differ in the spectra of cytokines they produce when stimulated by antigens.[13] The production of Th2 cytokines (IL-4, IL-5) by CD4+T lymphocytes is essential for the development of measles antibody production.[9,14] Th1 cytokines (IFN-γ, IL-2) also affects both B and T cell functions and plays a significant role in the control and resolution of measles virus infection.[15-17] Moreover, IFN-γ is critical for macrophage activation, T cell proliferation and differentiation and for up-regulation of human leucocyte antigens.[18-20] IFN-γ exerts an antiviral effect by promoting the lysis and clearance of measles-infected cells and by inhibiting viral gene expression and replication.[17]

The present study estimated IFN-γ levels among measles vaccinated children aged 1-10 years who had received a single or in some cases double dose of measles vaccine.

Materials and Methods

The study population consisted of 30 children selected according to specific selection criteria from the Vaccination Centre, Dept. of Virology, BSMMU. Of these, 24 children were vaccinated with one dose of measles vaccine at nine months of age according to the Expanded Program on Immunization (EPI) schedule, and 6 children were vaccinated with a second dose of measles vaccine during the Measles Catch-up Immunization Campaign. The relevant information and vaccination history from the study children were recorded in a standard pre-designed questionnaire/ data sheet by interviewing the guardians of the subjects. Informed written consent was taken from them before collection of blood samples.

For detection of IFN-γ level, measles vaccine virus was cultured and titrated at the Institute of Public Health (IPH), Mohakhali, Dhaka. Briefly, 1.5×10^6 Vero cells in Dulbecco Minimum Essential Medium (DMEM) culture medium containing 5% fetal calf serum was seeded into each of eight 75-cm^2 tissue culture flasks and incubated at 37°C in a 5% CO_2 humidified incubator until 70 to 80% confluent (approximately 3-4 days). Then, the Vero cells were infected with 3 mL of measles virus (Edmonston vaccine strain) and incubated at 37°C in a 5% CO_2 humidified incubator for 24–36 hour until the cells formed into an approximately 80–90% syncytia. Titration of measles vaccine virus was done by using the Spearman-Karber method of 50% tissue culture infective dose ($TCID_{50}$) and it was used as standard live virus.[21]

Fresh blood (5 ml) was collected in heparin and peripheral mononuclear cells (PBMC) were separated by Ficoll-Hypaque density gradient centrifugation technique at the Dept. of Virology, BSMMU. PBMCs were stimulated with both measles virus antigens and mitogens (lectin). Stimulated PBMCs were cultured in CO_2 incubator for 96 hours and IFN-γ level was measured from the culture supernatant by ELISA technique using the Human IFN-γ Quantikine Immunoassay (R&D Systems).

Statistical analysis: Data were analyzed by using SSPS software 15.0. Unpaired t test was done as required.

Results

The levels of Interferon gamma (IFN-γ) secretion by peripheral blood mononuclear cells (PBMC) due to different types of stimulation in children of various age groups who received a single dose of measles vaccine observed that the level of IFN-γ increased gradually with increasing age of the children. After stimulation with measles antigen, the IFN-γ level was lowest (12.97±8.16 pg/ml) in the 13-24 months age group, and reached its highest (105.00 pg/ml) level in the 109-120 months age group. Similarly, after lectin stimulation, the level of IFN-γ was also observed to be lowest (13.16±8.0 pg/ml) in the 13-24 months age group, and also peaked (226.54 pg/ml) in the 109-120 months age group (Fig 1).

Among the study children, 24 (80%) children received a single dose of measles vaccine while 6 (20%) children received two doses. After stimulation with measles antigen, the IFN–γ level in children who received two doses of measles vaccine was significantly higher (228.47±120.11 pg/ml) than those who received one dose of vaccine (62.38±50.51 pg/ml) (p<0.001). Similarly, after lectin stimulation, the IFN–γ level in children who received two doses of measles vaccine was significantly higher (184.5±80.90 pg/ml) than those who received only one dose of vaccine (69.85±45.14 pg/ml) (p<0.043) (Table I).

The association between nutritional status and IFN–γ level among the study population showed that the majority 27 (90%) were well-nourished while 3 (10%) were malnourished. After stimulation with measles antigen, IFN–γ level secreted by PBMC was higher (103.92±80.04 pg/ml) in well-nourished than in malnourished (94.67±50.14 pg/ml) children (p<0.900). Similarly, after lectin stimulation, IFN–γ level was higher (120.10±90.05 pg/ml) in well-nourished than in malnourished (89.74±45.56 pg/ml) children (p<0.698) (Table II).

Fig 1: Interferon gamma secretion by peripheral blood mononuclear cells (PBMC) by different types of stimulation in vaccinated children.
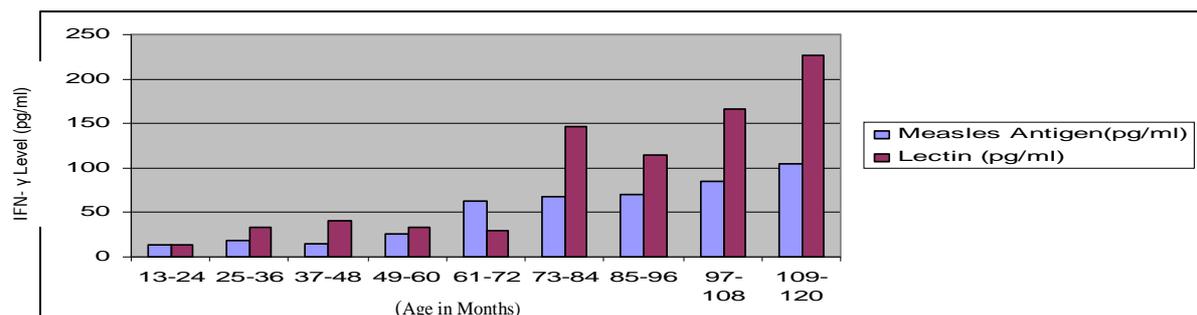
Table I: Association between numbers of doses of measles vaccine with level of Interferon-γ after stimulation.

Number of doses	Number of children (n=30)	Mean (±SD) Interferon gamma level (pg/ml) after stimulation with		
		Measles Ag	Lectin	No stimulation
1	24	62.38±50.51	69.85±45.14	8.79±4.45
2	06	228.47±120.11	184.51±80.90	17.03±5.32
P value		0.001	0.043	0.237

Unpaired t test was done.

Table II: Association between nutritional status and IFN-γ level after antigen stimulation.

Nutritional status	Number of children (n=30)	Mean (±SD) Interferon gamma level (pg/ml) after stimulation with		
		Measles Ag	Lectin	No stimulation
Well nourished	27	103.92±80.04	120.10±90.5	10.92± 6.05
Mal nourished	03	94.67±50.14	89.74±45.56	06.08 ± 4.10
P value		0.900	0.698	0.607

Unpaired t test was done.

Discussion

The introduction of an effective live attenuated virus vaccine has dramatically reduced the incidence of measles. Despite this, measles has not been eliminated because of failure to vaccinate all children during the first year of life and also due to cases of vaccine failure. Since the introduction of live attenuated measles vaccines, a gradual decline in the level of measles antibodies over time after immunization has been described but no cases of measles has been reported during follow-up.[22-26] These observations suggest that low titers of measles antibodies may be protective or that persistence of cellular immunity to measles may play an important role in preventing measles infection. Moreover, it has been established that individuals with sufficiently low antibody levels are at risk of mild or sub clinical measles infection.[27] It is thus important to understand the host response to measles vaccination among children in order to provide effective protection during the first year of life.

Our study observed that IFN-γ increased gradually with increasing age of children, being lowest in the 13-24 months age group and highest among 109-120 months after stimulation with both measles antigen and lectin. Measles immunization leads to the development of a measles virus specific memory T cells that may sustained for a prolonged period after immunization.

Children who received two doses of measles vaccine had high IFN-γ levels than those who received a single dose of measles vaccine. Although there are few precise data on the effect of a second dose of measles vaccination among children first immunized after one year of age, epidemiological studies show that increased protection is obtained after a second dose.[28] In an outbreak investigation in the USA in 1985, attack rates were 30%-60% lower in children who received two doses of measles vaccine compared with single-dose vaccinees.[29-30] This indicates that cell mediated immunity can be enhanced after a booster dose of measles vaccine given in the presence of high antibody titers. The cellular immune responses may be better sustained than antibody titers after vaccination and revaccination in some subjects.[5]

Malnutrtion can impair an individual's immune response to immunization with live attenuated vaccine like B.C.G, yellow fever, poliomyelitis vaccine. It may also affect the host response to measles vaccine. The impairment of some components of the immune system poses a theoretical possibility. Our study observed that the IFN-γ level was comparatively higher in well-nourished children than malnourished children but this difference was not statistically significant. Other studies have reported profoundly impaired T cell function in human malnutrition.[31-36] However, our study demonstrated that malnourished children responded adequately to measles vaccine.

It can be concluded from our study that IFN-γ level increased with increasing age of children, with higher IFN-γ level in children receiving a second dose of measles vaccine in comparison to children who received a single dose. Furthermore, our study did not observe any difference between malnourished and well-nourished children after measles antigen or lectin stimulation. Further extensive studies with larger sample size should be undertaken to detect IFN-γ responses after measles vaccination in different age groups to confirm the findings of our study.

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