The Efficacy of Bacillus Calmette-Guérin Vaccinations for The Prevention of Acute Upper Respiratory Tract Infection in The Elderly

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ABSTRACT

Aim: *to study the efficacy of BCG vaccinations, once a month for 3 consecutive months, in elderly on the prevention of acute upper respiratory tract infection (AURTI), interferon - gamma (IFN-γ) and interleukin (IL)-10 level in the BCG and placebo group and their comparison in the period of the study.*

Methods: *an original, experimental, prospective study with randomly pre-test and post-test design. Subjects were 60 – 75 years old, divided into BCG and placebo groups. The subjects in the BCG group received BCG vaccination, meanwhile the subjects in the placebo group received solvent for BCG vaccine, once a month for 3 months in succession at the deltoid region for each group. The subjects in both groups were evaluated every 2 weeks for the infiltrate diameter, on the 8th and 12th week for the scar measurements, and every 4 weeks for the prevalence of AURTI. The levels of IFN-γ and IL-10 were done at the initial and the end of the study. Data obtained were the prevalence of AURTI, the infiltrate and scar diameters at the site of BCG vaccination, and the levels of IFN-γ and IL-10. Data collected from January 2010 to June 2010 (6 months) at Internal Medicine Policlinics, Prof.Dr.RD Kandou General Hospital, Manado, North Sulawesi, Indonesia. Statistical analysis was performed using descriptive subject's characteristics, paired and un-paired T test and non-parametric test (Mann-Whitney and Wilcoxon test), and Spearman non-parametric test.*

Results: *in comparison between two groups in the period of the study, we found a significant reduction in the BCG group on the prevalence of AURTI and significant increase of IFN-γ level in BCG group compared to the placebo group. There were significant increase of IFN-γ and IL-10 levels in the BCG group compared to the placebo group. There were insignificant increase of IFN-γ and IL-10 levels in the pre- and post-BCG group. The increase of IFN-γ level was positively significant correlated with the increase of the infiltrate and scar diameters in the BCG group.*

Conclusion: *BCG vaccinations in elderly, once a month for 3 consecutive months, significantly prevent the AURTI and can increase the IFN-γ level as Th1 response and IL-10 as Treg response in the period of the study.*

Key words: *BCG, elderly, acute upper respiratory tract infection.*

INTRODUCTION

Aging is a process of the slow disappearance of tissues restoration capability to keep structures and functions normally from any traumas, including infections. In many countries in Europe and The United States, the definition of the elderly is for those who are more than 65 years old, meanwhile in Indonesia the definition is subjects according to the World Health Organization (WHO) in 1989, for those who are more than 60 years old, where many organ systems are declining in functions, including the immune function, since the age of 30 – 40 years old and the progressivity of declining is 1% per year.[1-3]

There is a change in elderly population in The United States and other industrial countries where the elders are growing in number from 12 million in 1950 (8% of total population) to 36 millions in 2002 (12% of total population), since the life expectation also increases from 71.1 years in 1950 to 79.9 in 2002, and the influenza and pneumonia are the fifth most cause of death among the elders.[4] In Indonesia, from the medical records at Dr. Karyadi General Hospital Semarang, the respiratory tract infection is the second most cause of morbidity and mortality among the elders, caused by the declining of immunity status in elderly.[5]

The immune system is changes with the ageing process, which is known as immunosenescence. The

most significant change of the immune system is the reduction of immune protection against infections caused by the complex interaction between primary immune defect and homeostatic compensation mechanism resulting in disturbance in the regulation of optimal and effective immune function.[6]

The thymus is a primary lymphoid organ as the place for T lymphocytes to become competent. The thymus itself will reduce in size, from the age of 9 months old and in the age of 45-50 years old the size is 5-10%, changed by fat which may reduce the number of CD3+, CD4+, and CD8+ cells.[4,7,8] The primary changing of cellular immunity is signed by the reduction of T naïve cells and the increasing of memory T cells in numbers as the result of the reduction of IL-2 level, high affinity of IL-2 receptors, and reduction of T cell responses to antigens. The increasing of memory T cells may cause the increasing on the production and secretion of IL-10 which may inhibit the production of IL-12 and IFN-γ.[4,9] In the elderly, the cytokines production from T helper (Th)2 cells (IL-4, IL-5, IL-10, and IL-13) is higher, meanwhile the production of Th1 cells derived cytokine are decreased.[9,10] Humoral immunity response is also reduced in the elderly. The activation and proliferation of B cells are disturbed, lowering production of antibodies in quantity, quality, and the affinity of antibodies is reduced. The reduction in number of CD4+ T cells to back up the B cells activation and differentiation may reduce the antibodies productions as the responses to antigen exposures. The reduction of the immune system capability to protect the body from the pathogenic microorganisms infection will reduce the responses to infection and the vaccinations.[7,9,11,12]

The BCG vaccine is a vaccine containing the weakened living Mycobacterium bovis, to prevent tuberculosis diseases, but it is also used as immune stimulant in other conditions. This vaccine was used orally for the first time in 1921, then by the intra-dermal distribution (0.1 ml) at the deltoid region due to the better induction in late type hypersensitivity and lower in cost. If the BCG vaccination is given just for once, then the stimulus to the immune system is too slow, weak, and rapid compared to the stimulus coming from tuberculosis infection.[13,14] The reactions that are happening after the BCG distribution may include the local reactions, such as: induration, erythema, pustule, and resulting in ulcer and will heal spontaneously in 8-12 weeks, leaving a scar. There is also a possibility for regional reaction (axillary lymphadenopathy) which will heal spontaneously in 3 months.[14,15]

The mycobacterium is one of the potential immunomodulator microorganism and will give strong effect to the cytokines environment in the lungs. The BCG (containing 45-mer oligodeoxynucleotide or CpG ODN) is chosen in immunomodulation system because it may significantly induce the increasing of IFN-γ.[16,17] The difference between those who already had BCG vaccinations and those who did not have BCG vaccination is the scar at the site of BCG vaccination (80-90%). This scar may appear in eigth weeks and may last until 3 years after the BCG vaccination, with 2-8 mm in diameter.[18-20] The post-BCG vaccination's infiltrate and scar may be a sensitive indicator of effective vaccination. The protein from BCG vaccine will be processed and presented by dendritic cells to T cells through human leukocyte antigen (HLA) class II. The monocytes will come out from blood vessels into the site of vaccination and may cause edema, induration, infiltrate, and finally the scar. The post-BCG vaccination scar refers to the higher proportion of cytotoxic T cells and IFN-γ.[19-21] In people with Th2 derived cytokines responses, the BCG vaccination will increase the Th1 cells derived cytokines and IFN-γ levels and it will make the post-BCG vaccination scar as a production parameter of Th1 cells derived cytokines especially IFN-γ.[22,23] The failure of forming the post-BCG vaccination scar may be caused by the less immune maturity, the wrong injection technique, and the bad quality of the vaccine.[18,19]

METHODS

This study is an original experiment, with a random pre-test and post-test design. The subjects are 60-75 years old from Internal Medicine Outpatient Department, Prof. Dr. RD Kandou General Hospital, Manado, North Sulawesi, Indonesia. The target population was those who fulfilled the inclusion criteria, age ≥ 60 years old (World Health Organization/ WHO criteria 1989), agreed to participate, and sign informed consent. The exclusion criteria were those who had history of bronchial asthma, emphysema, pulmonary tumor, pneumonia, and other pulmonary diseases or abnormalities (based on anamnesis, physical examinations, chest X-rays, less than 80% best value of peak expiration flow in 3 times of measurements using mini Wright peak flow meter from Clement Clark International Ltd England with the catalog number: 3130057), diabetes mellitus (PERKENI concensus 2006), chronic kidney disease (National Kidney Foundation 2003), liver function disturbance (reversed ratio of albumin-globulin and

SGOT and SGPT more than two folds of normal values), clinically had rheumatoid arthritis, systemic lupus erythematosus, polymiositis, and auricularis chondritis, protein malnutrition (albumin level less than 3.5 g/dl), carcinomas, active smokers, and were had immunotherapy and or BCG vaccinations previously prior to or during the study, refusing informed consent, not following the procedures, and moved out of town or dying during study.

After all the subjects had signed the informed consent, they were randomly divided into BCG and placebo groups. All subjects in the BCG group had an 0.1 ml of BGG vaccination (weakened living Mycobacterium bovis Pasteur Paris strain mp 1173-P2 produced by PT Biofarma, Bandung, Indonesia- dried form of vaccine in every vial diluted by 2 ml of solvent – 0.1 ml for one dose of intra-dermal injection) once a month for 3 consecutive months at the deltoid region using 1 ml disposable syringe and 26 G needle. All subjects in the placebo group had 0.1 ml of the solvent of the BCG vaccine as placebo for one dose of intra-dermal injection once a month for 3 consecutive months at the deltoid region using 1 ml disposable syringe and 26G needle. Initial chest X-rays, the peak expiratory flow examinations, and blood samples were obtained to measure the levels of fasting blood sugar, SGOT and SGPT, albumin, globulin, serum creatinin, IFN-γ, and IL-10. Both groups were evaluated every 2 weeks for the diameter of infiltrates, on the 8th and 12th week for the scar measurements at the site of vaccination in the BCG group, and every 4 weeks for the prevalence of AURTI in all subjects. After 6 months, the final blood samples were obtained to measure the level of IFN-γ and IL-10 levels in all subjects in both groups.

The data obtained were revealed as text, figure, and tables. Statistical analysis was performed using descriptive for subject's characteristics, paired T test for normal data distribution or the Wicolxon non-parametric test for abnormal data distribution to compare the IFN-γ and IL-10 levels before and after treatment in the BCG group, un-paired T test for normal data distribution or the Mann-Whitney non-parametric test for abnormal data distribution to compare the prevalence of AURTI, the levels of IFN-γ and IL-10 in both groups, and Spearman non-parametric test for the correlation between the increased of IFN-γ level and the increase of diameter of infiltrates and scars in the BCG group after 6 months of BCG.

RESULTS

From data collected between June 2009 and November 2009, 36 subjects were recruited, but 2 subjects dropped out because of moving out of town or refused to continue to participate in the study. At the end of this study, 34 subjects (26 women and 8 men) were divided into the BCG and placebo groups to complete the study for the whole 6 months. All of the subjects were 60 – 75 years old. The normality test of data was performed for the prevalence of AURTI during the study was revealed with normal distribution ($p<0.05$) in both groups. The normality test for IFN-γ and IL-10 levels were revealed with the results of normal distribution ($p<0.05$) for IFN-γ and IL-10 levels at the initial of the study, and IFN-γ level at the end of the study in the BCG and placebo groups. The normality test for the diameter of infiltrates and scars in the BCG group (between the initial and the end of the study) was revealed with the results of normal distributions ($p<0.05$) on infiltrates for the second BCG vaccination (at the 8th and 10th weeks) and scars for the first BCG vaccination (at 12th week).

During 6 months period of the study, the BCG group had 2 times of AURTI, meanwhile the placebo group had 3 times of AURTI. The statistical difference tests on IFN-γ and IL-10 levels at the initial and end of the study in the BCG group, and IFN-γ and IL-10 levels at the initial and end of the study in the BCG and placebo groups are presented in **Table 1**. The increased and statistical test of the infiltrates and scars in the BCG group are presented in **Table 2**. The correlation between the increased of IFN-γ level and the increase of diameter of infiltrates and scars in the BCG group are revealed with the result that the increase of IFN-γ level will be followed by the increased of infiltrates and scars diameter ($p<0.05$).

Table 1. The difference tests of IFN-γ and IL-10 levels

A. At the initial compared to end of the study in the treatment group			
	Initial	**End**	**p**
IFN-γ (median)	0.55 pg/ml	0.07 pg/ml	0.065
IL-10 (median)	0.27 pg/ml	0.29 pg/ml	0.127
B. The comparison between treatment and control groups at the end of the study			
	Treatment	**Control**	**p**
IFN-γ (median)	0.07 pg/ml	0.04 pg/ml	0.007
IL-10 (mean)	0.30 pg/ml	0.27 pg/ml	0.043

DISCUSSION

There were 34 subjects who were involved in this study, divided into BCG and placebo groups, each group consisting of 13 women (76.5%) and 4 men (23.5%). The rate of age in the BCG group was 64

Table 2. The increased in diameter of infiltrates and scars followed by the increased of IFN-γ level in the treatment group

Variables		Week	After BCG vaccinations		
			Median (pg/ml)	SD	p
Infiltrates					
Vaccination	BCG I	2	9.500	2.750	<0.05
		4	7.500	2.592	<0.05
		6	6.500	2.035	<0.05
	BCG II	6	12.000	2.996	<0.05
		8	9.000	2.942	<0.05
		10	8.000	2.518	<0.05
	BCG III	10	13.500	3.873	<0.05
		12	11.000	3.701	<0.05
		14	9.000	3.189	<0.05
Scars					
Vaccination	BCG I	8	5.500	1.810	<0.05
		12	4000	1.222	<0.05
	BCG II	12	6.500	1.881	<0.05
		16	5.500	1.456	<0.05
	BCG III	16	7.500	2.348	<0.05
		20	6.500	1.863	<0.05

years old, and in the placebo group was 66 years old. Statistical analysis on age and sex showed that there was no significant difference between the two groups.

The immune system will change with the increased of age that the cellular and humoral immunity responds to antigens are reduced, but the responses to auto-antigens will increase. The aging process on the immune system will disturb the regulation of the immune system that will produce the fragility caused by the infections, cancers, and auto-immunity diseases.[1,6,24,25] The changes on immunity in elderly also related to the reduce of responses to influenza virus infection and immunizations.[26]

The influenza and other respiratory tract infections are the main causes of hospitalization and mortality in elderly. In the United States, there is a prediction that about 90% death per year from 100,000-400,000 elderly are caused by influenza. The chronic diseases, such as diabetes mellitus, kidney disturbance, and any other chronic diseases, will make the risk of the infection into 40-150 times higher.[26] The increasing prevalence of influenza in elderly is caused by the decrease of cellular immunity and antibody response to influenza vaccination. The cytotoxic T cells play an important role in the clearance of influenza virus from the lungs. In the aging process, there is a switch of cytokines, from Th1 derived cytokines (especially IFN-γ) into Th2 derived cytokines (IL-10) which are related to the activity reduction of cytotoxic T lymphocyte cells and it may cause the reduction of protection from influenza virus infection. The viral specific T lymphocyte cells are found in the infected lungs due to the Th1 cells response, specifically by the production of IFN-γ. The influenza specific Th2 derived cytokines (including IL-10) are present in high levels at the site of infection, producing the imbalanced between Th1 and Th2 cells derived cytokines.[13,25] In this study, after 6 months observation and physical examination from nose, throat, and chest by inspection, palpation, percussion, and auscultation, it was found that the most prevalence of AURTI is rhino-pharyngo-laryngo-tracheitis. The prevalence of AURTI in the elderly who had BCG vaccinations is only in 2 subjects (8.7%) in the 4th week of the study and they were all treated by symptomatic treatments and vitamin, meanwhile the AURTI happened in whole weeks of the study in the placebo group. In the placebo group, there were 10 subjects (91.3%) who had AURTI and 8 of them had repeated AURTI ($p<0.05$). The AURTI in the BCG group might happen because there was not enough level of IFN-γ to increase the cellular immunity against the virus. None of the subjects from the BCG group had serious complication from the influenza during the study, and they were all further included in the study.

In several studies, The BCG vaccination was found to increase the expression of Th1 cells to produce IFN-γ and IL-2. Choi, et al in Korea, found that a single BCG vaccination might induce Th1 responses in asthma patients who are maintained under sub optimal medications might be overtaken after 12 weeks by underlying Th2 type disease, and increases in IFN-γ/IL-4 ratio at week 4, which were the reason in this study giving BCG vaccination once a month for 3 consecutive months, to strengthen the Th1 response.[27] Wongdjaja K in Manado, found that by BCG vaccinations once a month for 2 and 3 months period in succession would increase the level of IFN-γ.[28] Datau and Surachmanto EE29 in Manado, found that by 3 times of BCG vaccinations to bronchial asthma patients would increase the level of IFN-γ started from 12 weeks after the BCG vaccinations and reached the significant increase of IFN-γ in 6-9 months after the BCG vaccinations. In this study, the increase of IFN-γ was statistically significant ($p<0.05$), compared to the placebo group, with the median increased of IFN-γ was 0.03 pg/ml. There was also an insignificant increased of IL-10 level in 6 months after BCG vaccinations compared to the initial time in the BCG group. The level of IFN-γ was insignificant increased after BCG vaccinations compared to the initial of the study in

the BCG group (p>0.05).There was also a significant increased in the mean of IFN-γ level (p<0.05), although it was not as high as the results from Wongdjaja and Datau studies in extrinsic atopic bronchial asthma (0.67 pg/ml and 1.03 pg/ml). This difference was due to the use of younger subjects than the subjects in this study and the different mechanisms between bronchial asthma and elderly. In the elderly, the low response to BCG challenge is caused by intrinsic production deficiency of IFN-γ due to the reduction of naïve T cells and the increase of memory T cells in the thymus involution. Although the IFN-γ is the important element from protection response, the total IFN-γ level should not be used as the only parameter reflecting the immune response. Other mechanisms from immune system, especially CD8+/cytotoxic T cells also contributes to the protection process by supporting additional source for IFN-γ to induce the apoptotic pathway that has a role in the process of microorganism, destruction, or by the destruction process of the infected macrophages.[28-30]

The possible explanation for the insignificant increase in IL-10 levels was due to the identified bacterial DNA component, the CpG ODN, induced the expression of several cytokines genes, including IFN-γ, but not so high to suppress IL-10. The CpG ODN will directly activate the antigen presenting cells (APC) consisting of macrophages and dendritic cells. The activated macrophages then produced IL-12, IL-18, TNF-α, IFN-α, and β.31These cytokines will stimulate the production of IFN-γ from natural killer (NK) cells and Th1 cells. On the other hand, the IFN-γ will activate the macrophages through positive feed-back to produce IL-12 and IL-18. Like the macrophages, the dendritic cells are the main APC in the lungs that has an important role in initiating the immune response. The CpG ODN will stimulate the dendritic cells to produce IL- 6, IL-12, and TNF-α, strengthen the lymphocytes activities, inducing the production of Th1 cells derived cytokines. The influence of IL-12 will make the differentiation of T cells to the Th1.[31] The dominance of Th1 cells will change the balance of T regulators or Treg cells (producing tissue growth factor or TGF-β and IL-10), Th17 cells (producing Il-17 and IL-6), Th1 cells, and Th2 cells in the balancing square model.[32] Using the balancing square model, the people with the Th2 cells dominant (e.g. the atopic people), through the BCG, the Treg cells will be activated to suppress the Th2 cells activity then the Th1 cells will increase as the counterbalance to the Th2 cells suppression, and the Th17 cells will be inactivated. This process will keep the IFN-γ at considerably high level and protect the elderly from viral infections, including influenza (**Figure 1**).[32]

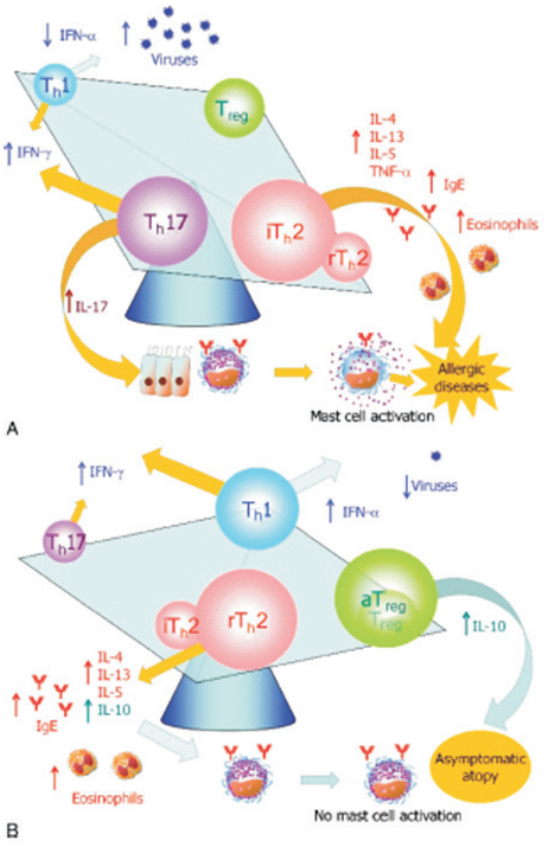

Figure 1. Onset of allergic diseases may be determined by the ratio of proinflammatory T-cell subsets (Th17 and iTh2) versus Treg subsets (balancing square model). A, In patients with chronic allergic diseases, proinflammatory T-cell subsets, that is, Th17 cells and Th2 cells that are capable of producing high levels of TNF-γ (iTh2 cells) are upregulated. B, In asymptomatic atopic individuals, Th2 cells that are capable of producing IL-10 (rTh2 cells) may be up-regulated, and Th17 cells may be inactivated.[32]

The post-BCG vaccination scar is usually used as the parameter of effectively vaccination.[19] Local tissue reaction at the side of BCG vaccination is equal to the production of IFN-γ as the response to micro-bacterial antigens, and it was found in 99.1% as infiltrate in Brazil and in 92.5% as scar in India. Intra-dermal administration of BCG vaccination significantly will induce the increase of Th1 cells derived cytokines, especially IFN-γ that will inhibit the Th2 immune response.[18,33] In this study, there were post-BCG vaccinations as infiltrates and scars in all of subjects in the BCG group, but none was found in subjects of the placebo group. The diameter of infiltrates and scars were bigger related to the number of BCG vaccinations they had. The increase of IFN-γ would follow by the increase of the diameter of post-BCG vaccinations infiltrates and scars. The increase of serum IFN-γ levels, the reduction on the prevalence of AURTI, and the form of post-BCG vaccination infiltrates and scars after 3

times of BCG vaccinations which were not found in the placebo group, are the figures of the switching process from Th2 cells to Th1 cells dominant in the elderly after 3 times of BCG vaccinations.

CONCLUSION

The BCG vaccinations in the elderly, once a month for 3 consecutive months, may give effective immunity protection as prevention of AURTI by increasing the Th1 respond, shown by the increase of IFN-γ level, reflecting through the formation of infiltrates and scars at the site of BGC vaccinations.

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