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CD4+ AND CD8+ T-CELLS EXPRESSING INTERFERON GAMMA IN ACTIVE PULMONARY TUBERCULOSIS PATIENTS

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Abstract

Background: Tuberculosis (TB) is a global health problem. Immune response through $CD4^+$ and $CD8^+$ T cells is needed to produce Interferon gamma (IFN-γ), a cytokine eliminate *Mycobacterium tuberculosis*. We aimed to compare the cellular immune response based on the percentage of $CD4^+$ and $CD8^+$ T cells expressing interferon gamma in active pulmonary tuberculosis patients before and after 2 months of tuberculosis treatment.
Methods: It is a longitudinal cohort study included 12 patients with new active pulmonary TB of the Pulmonary Hospital, Surabaya. The $CD4^+$ and $CD8^+$ T cells expressing interferon gamma was measured by flow cytometry method.
Results: The mean $CD4^+$ interferon gamma percentage of new active pulmonary TB before treatment was higher than 2 months after tuberculosis treatment (4.48% vs. 1.52%) and there was a significantly decreased (p = 0.025). The mean $CD8^+$ interferon gamma percentage of new active pulmonary TB before treatment was higher than 2 months after tuberculosis treatment (3.56% vs. 2.89%) but not significantly decreased (p = 0.186).
Conclusions: The mean $CD4^+$ IFN-γ percentage of new active pulmonary TB before treatment was higher than 2 months after treatment, suggesting that $CD4^+$ T cells expressing IFN-γ play a role in protection against pulmonary TB infection.

Keywords: IFN-γ, $CD4^+$, $CD8^+$, new active pulmonary tuberculosis, Flow cytometry, OAT

Introduction

Tuberculosis (TB) is still one of the deadly transmitted disease worldwide. Approximately 9 million people suffered and 1.5 million died from tuberculosis in 2013. More than half of 9 million (56%) people suffering from tuberculosis live in South East Asia and West Pacific with three hundred and sixty thousand of those are Human Immunodeficiency Virus (HIV) positive. Although new TB cases are gradually decreasing every year, it is the cause of million people's illness every year and the second cause of death after HIV infection (Philippe, et al., 2014). About 60% of TB cases with high mortality occurred in males, but females also have high a burden of disease. In 2013, about 510.000 females died from TB and more than one third of them were HIV positive. At the same year, there was 80.000 mortality because of TB in children with HIV negative (WHO, 2014).

TB infection is still a health problem in Indonesia. According to WHO 2013, the incidence of TB in Indonesia is ranked fourth (410-520 thousand) after India (2-2.3 million), China (0.9-1.1 million), and Nigeria (340-880 thousand). Indonesian Basic Health Research survey in 2013 revealed that the prevalence of TB based on diagnosis was 0.4% of the total population. Four hundred people in every 100,000 population was diagnosed as TB by health workers based on sputum examination, Chest X-ray or both during a period less than 1 year. These results are not different from the Basic Health Research survey in 2007 (WHO, 2014; Indonesian Health Ministry, 2014).

Long-term therapy at least 6 months with oral anti TB (OAT) drugs without compliance is not successfull to reduce the TB event and causes drugs resistance. Clinical, bacteriology, and radiology improvement in TB patients will be obtained in second month after effective therapeutic regimen of OAT. Sputum culture status in the second month is the only acceptable biomarker for assessing TB therapy response. Obtaining good quality of sputum, particularly in children with TB and extrapulmonal TB is difficult (Ivy et al.,2014; Mitchison, 1993).

The presence of *Mycobacterium tuberculosis* in macrophages will provide a signal, resulting in production of major histocompatibility complex (MHC) class II molecule by endoplasmic reticulum which carrying *M. tuberculosis* fragments to the surface of macrophage, then expressing it on the surface of macrophage in order to be recognized and

bound to CD4+ T-lymphocytes receptor. Activated $CD4^+$ T-lymphocytes will produce cytokines which are important for destroying or controlling the growth of *M. tuberculosis*. The main cytokines are interferon gamma (IFN-γ), TNF-α and IL-2. IFN-γ strengthens the potential phagocyte of infected macrophage through formation of phagolysosom. IFN-γ also stimulates the formation of free radicals which are able to destroy the component of *M. tuberculosis*. $CD8^+$ T-cells could produce IFN-γ for destroying *M. Tuberculosis*, along with $CD4^+$ T-cells to control the growth of *M. tuberculosis*. IFN-γ will stimulate macrophages that containing the bacteria to increase reactive nitrogen intermediate needed to destroy *M. Tuberculosis* (Abbas et al., 2014; Alamelu, 2004).

Siri Laura Feruglio *et al* (2014) confirmed that the total percentage of IFN-ɣ secreted by $CD4^+$ T-cells and $CD8^+$ T-cells decrease after 2 months receiving anti TB therapy. Thus become a strong indicator of successful TB treatment, as well as a biomarker for potential effectiveness of TB therapy (feruglio et al., 2014). In addition, the percentage of IFN-ɣ secreted by CD4+ and CD8+ T-lymphocytes in TB patients at the time of diagnosis was significantly higher than control and back to the normal level after OAT therapy (Hanne et al., 2007).

High incidence of TB with the clinical, bacteriology, and radiology limitation background which affect the TB therapy success rate during 2 months intensive phase encouraged the researchers to conduct this research. This research compared the host cellular imune response to TB infection, particularly the percentage of $CD4^+$ and $CD8^+$ T-cells expressing interferon-gamma in active lung TB patients before and after 2 months intensive phase of OAT therapy.

The purpose of this study was to prove the therapeutic effect of 2 months OAT therapy to cellular immune response in terms of decrease in percentage of $CD4^+$ and $CD8^+$ T-cells which express interferon-gamma in new patients with active lung TB. This study is expected to be useful for assessing the success in response of 2 months OAT therapy in new patients with active lung TB.

Materials and Methods

It is a longitudinal cohort study with comparison in a population with new active pulmonary TB before and after 2 months receiving OAT therapy. The patients received OAT regimens consisted of Rifampicin, Isoniazid, Pyrazinamide and Ethambutol. This study was conducted in January–May 2015. Samples were obtained from new active pulmonary TB patients before and after 2 months receiving OAT therapy from a specialty lung disease hospital Surabaya with inclusion criteria: Aged 21 years old or more; The patients have diagnosed as active lung TB based on clinical signs and symptoms, Chest X-Ray, and positive result of Acid Fast Bacilli examinations and the patients have never been treated with OAT previously. We excluded the patients who received immunosuppressant therapy or suffering from immunodeficient or immunosuppressive diseases like HIV infections, diabetic and malignancy and the patients with active lung TB combined with another lung diseases. Written informed consent was obtained from all participants and the study protocol was approved by the ethics committees of Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia (No.128/EC/KEPK/FKUA/2015).

Percentage of CD4+ T-cells, CD8+ T-cells, and both of CD4+ and CD8+ T-cells expressing IFN-γ were examined at the Clinical Pathology Department, Dr. Soetomo Hospital Surabaya. Sample size was 12 patients with new active pulmonary TB and followed until the end of intensive phase therapy (2 months of OAT therapy). Percentage of $CD4^+$ and CD8+ T-cells which express IFN-γ were measured from *peripheral blood mononuclear cells* material of the new active pulmonary TB patients before and after 2 months OAT therapy using flowcytometry method, *FACSCalibur*.

Difference in percentage of $CD4^+$ and $CD8^+$ T-cells expressing IFN-γ in new active pulmonary TB patients before and after 2 months OAT therapy were analyzed using paired T-test. Decreasing of $CD8^+$ and $CD4^+$ T-cells expressing IFN-γ in new active pulmonary TB patients before and after OAT therapy were analyzed using Wilcoxon Signed Rank Test. Statistical analysis was performed by SPSS, version 19 (SPSS Inc., Chicago, IL, USA), $p < 0.05$ was considered statistically significant.

Results

The participants comprised 12 new active pulmonary TB patients which were followed from the beginning until the end of two months of OAT therapy.

Table 1: Characteristic of samples based on sex

Sex	Frequency	Percentage (%)
Males	3	25
Females	9	75
Total	12	100

Table 2: Characteristic of samples based on age

Age (years old)	Frequency	Percentage (%)
≤30	2	16.7
31-40	2	16.7
41-50	5	41.7
>50	3	25.0
Total	12	100.0

$CD4^+$ T-cells percentage in the group with new active pulmonary TB before OAT therapy was 31.44–74.49%, the mean was 48.79% with standard deviation 12.45%. $CD4^+$ T-cells percentage in the group with new active pulmonary TB after OAT therapy was 31.86–60.89%, the mean was 44.85% with standard deviation 8.50%. Paired samples t-test analysis revealed that the decrease of $CD4^+$ T-cells percentage after 2 months OAT therapy was not statistically significant (p >0.05).

Table 3: Percentage of $CD4^+$ T-cells before and after 2 months OAT therapy

	N	$CD4^+$ T-cells (%)				*p*
		Mean	SD	Min	Max	
Before therapy	12	48.79	12.45	31.44	74.49	
After 2 months therapy	12	44.85	8.50	31.86	60.89	**0.224**

$CD8^+$ T-cells percentage in the group with new active pulmonary TB before OAT therapy was 22.35–62.62%, the mean was 38.66% with a standard deviation of 10.16%. $CD8^+$ T-cells percentage in the group of new active pulmonary TB after OAT therapy was 22.55–63.90%, the mean was 36.52% with standard deviation 9.76%. *Wilcoxon Signed Ranks Test* analysis revealed that the decrease of $CD8^+$ T-cells percentage after 2 months OAT therapy was not statistically significant (p >0.05).

Table 4: Percentage of $CD8^+$ T-cells before and after 2 months OAT therapy

	N	$CD8^+$ T-cells (%)				*p*
		Mean	SD	Min	Max	
Before therapy	12	38.66	10.16	22.35	62.62	
After 2 months therapy	12	36.52	9.76	25.55	63.90	**0.154**

Percentage of $CD4^+$ T-cells expressing IFN-γ in the group with new active pulmonary TB before OAT therapy was 0.33–23.36%, the mean was 4.48% with standard deviation 6.24%. Percentage of $CD4^+$ T-cells expressing IFN-γ in the group with new active pulmonary TB after 2 months OAT therapy was 0.22–6.29%, the mean was 1.52% with standard deviation 1.79%. Wilcoxon Signed Ranks Test analysis revealed the decrease of CD4+ T-cells percentage expressing IFN-γ in new active pulmonary TB patient after 2 months OAT therapy was statistically significant (p <0.05).

Table 5: Percentage of $CD4^+$ T-cells expressing IFN-γ in new active pulmonary TB patients before and after 2 months OAT therapy

	N	$CD4^+$ T-cells expressing IFN-γ (%)				*p*
		Mean	SD	Min	Max	
Before therapy	12	4.48	6.24	0.33	23.36	
After 2 months therapy	12	1.52	1.79	0.22	6.29	**0.025***

* statistically significant with α = 0.05

Percentage of $CD8^+$ T-cells expressing IFN-γ in the group with new active pulmonary TB before OAT therapy was 1.09–8.67%, the mean was 3.56% with standard deviation 2.29%. Percentage of $CD8^+$ T-cells expressing IFN-γ in the group with new active pulmonary TB after 2 months OAT therapy was 0.99–6.79%, the mean was 2.89% with standard deviation 1.55%. Paired samples t-test analysis revealed the decrease of CD8+ T-cells percentage expressing IFN-γ in new active pulmonary TB patient after 2 months OAT therapy was not statistically significant (p >0.05).

Table 6: Percentage of $CD8^+$ T-cells expressing IFN-γ in new active pulmonary TB patients before and after 2 months OAT therapy

	n	$CD8^+$ T-cells expressing IFN-γ (%)				*p*
		Mean	SD	Min	Max	
Before therapy	12	3.56	2.29	1.09	8.67	
After 2 months therapy	12	2.89	1.55	0.99	6.79	**0.186**

Discussion

We revealed the proportion of female subjects was higher than males in this study. The similar result was found by WHO (2014) . In Africa and South East Asia region, the incidence of TB in females was 69% of all cases. Most of the TB patients in this study were 41-50 years old. It might be correlated with high mobility and social interaction as a productive worker which has posibility to be infected by other TB patients (WHO, 2014; Godoy et al., 2001).

Decrease percentage of $CD4^+$ T-cells in new active pulmonary TB patients after 2 months OAT therapy in this study was not significant. Mean percentage of $CD4^+$ T-cells was lower in new active pulmonary TB patients after 2 months OAT than before treatment that may associated with sequestration of $CD4^+$ T-cells at the site of infection in the lungs before being treated. Another reason is the reduction of bacterial load and inflammation during TB therapy causes decreasing $CD4^+$ T-cells at the site of infection and returning $CD4^+$ T-cells to the peripheral circulation (Dieli et al., 1999).

When *M. Tuberculosis* infection occurred in pulmonary tissue and adaptive immunity takes a role to eliminate the bacteria while innate immunity failed. *Naive* T-cells and $CD4^+$ in peripheral circulation will be distributed to site of the infection in the lungs, followed by recognizing and presentation of antigen to *naive* T-cells by dendritic cells and macrophage through MHC class II molecule, resulting a differentiation of *naive* T-cells to $CD4^+$ T-cells. After 2 months OAT therapy, activated $CD4^+$ T-cell on the site of infection undergoing apoptosis and $CD4^+$ T-cells that have not been activated return to the peripheral circulation along with *naive* T-cells (Feruglio, 2014). In this study, eventhough the percentage of $CD4^+$ T-cells was decreased after 2 months OAT therapy, it was not significant.

This result was different from the study of Feruglio et al. we found that the percentage of $CD4^+$ T-cells in new active pulmonary TB patient before and after 2 months OAT therapy was significantly different. However in contrast with this study, they also involved extrapulmonary TB patients (Feruglio et al., 2014). Other study stated that the frequency of activated $CD4^+$ T-cells in extrapulmonary TB patients was higher than pulmonary TB patients (Almeida et al., 2011). Decreasing antigen load after 2 months OAT therapy causes a decreasing mean percentage of $CD4^+$ T-cells in extrapulmonary TB patients more than pulmonary TB. Thus, it causes decrement percentage of $CD4^+$ T-cells after 2 months therapy become significant.

Statistical analysis revealed that the decreased percentage of $CD8^+$ T-cells in new active pulmonary TB patients after 2 months OAT therapy was not significant. Although mean percentage of $CD8^+$ T-cells in new active pulmonary TB patients after 2 months therapy was lower than before therapy. This result might be caused by sequestration of $CD8^+$ T-cells at the site of infection in the infected lung before therapy and returning to the peripheral circulation after therapy (Caccamo et al., 2009). Insignificant decreasing $CD8^+$ T-cells in patients after 2 months OAT therapy may be caused by repopulation of $CD8^+$ and *naive* T-cells in peripheral circulation so eventhough the mean percentage of $CD8^+$ T-cells was decreased but the result was not significant because the bacterial load was also decreased.

We revealed a significant decreased in percentage of $CD4^+$ T-cells expressing IFN-γ in new active pulmonary TB after 2 months of OAT therapy. This result was similar with several studies that showed decreasing bacterial load caused by OAT therapy effects with evidence of Acid Fast Bacilli sputum conversion becoming negative. Conversion of AFB sputum was in accordance with a decrease in percentage of $CD4^+$ T-cells expressing IFN-γ (Feruglio et al., 2014; Hanne et al., 2007).

The main effector function of $CD4^+$ T-cells is synthesizing IFN-γ and other cytokines which stimulated macrophages. Based on the study of Green, *et al.* IFN-γ must be synthesized by $CD4^+$ T-cells to provide a maximum protection from *M. tuberculosis* infection. IFN- γ from $CD4^+$ T-cells directly increase respons of $CD8^+$ T-cells to *M. tuberculosis* infection. $CD8^+$ T-cells was the main source of IFN- γ in mice with $CD4^+$ deficiency (Green et al., 2012).

We found that there was no significant decrease in percentage of $CD8^+$ T-cells expressing IFN-γ in new active pulmonary TB patients after 2 months of OAT therapy. Mean percentage of $CD8^+$ T-cells expressing IFN-γ in new active pulmonary TB patients after 2 months of OAT therapy was lower than before therapy although the difference was not significant. This result might be caused by effector function of $CD8^+$ T-cells in controlling TB infection which predominantly act as cytotoxic cells than IFN-γ secreted cells. Based on study of Dhruv Sud *et al*, maximum eradication of TB infection occurred after $CD8^+$ T-cells gradually secreting IFN-γ followed by cytotoxic activity of the cells (Dhruv et al., 2006). Even though percentage of $CD8^+$ T-cells expressing IFN-γ was decreased after 2 months of OAT therapy but was not significant compared with before therapy because effector function of $CD8^+$ T-cells in producing IFN-γ was not predominant in pulmonary TB. Decreasing percentage of $CD8^+$ T-cells expressing IFN-γ during intensive phase of OAT therapy did not show effectiveness of OAT therapy.

The role of $CD8^+$ T-cells in TB has not clearly explained just than $CD4^+$ T-cells, while $CD8^+$ T-cells contribute to provide an optimum protection and immunity. $CD8^+$ T-cells have a number of effector mechanisms against bacterial infection which is not found in $CD4^+$ T-cells (Teresa et al., 2014)

We only involved new active pulmonary TB group become limitation of this study. Further research is needed to study about the percentage of $CD4^+$ and $CD8^+$ T-cells expressing IFN-γ in extrapulmonary TB patients which could be used for monitoring extrapulmonary TB.

Conclusion

Although there was no decrease in percentage of $CD4^+$ and $CD8^+$ T-cells in new active pulmonary TB patients after 2 months OAT therapy, we found a decrease in percentage of $CD4^+$ and $CD8^+$ T-cells expressing IFN-γ in new active pulmonary TB patients after 2 months OAT therapy. $CD4^+$ and $CD8^+$ T-cells expressing IFN-γ could be used for monitoring treatment response during intensive phase of OAT in new active pulmonary TB patients.

Conflict of Interest: The authors declare that there is no conflict of interest in this study.

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References

1. Abul K. Abbas, Lichtman Andrew H. & Pillai Shiv (2014). Effector Mechanisms of T Cell–Mediated Immunity Functions of T Cells in Host Defense. *BASIC IMMUNOLOGY: FUNCTIONS AND DISORDERS OF THE IMMUNE SYSTEM.* Philadelphia USA: Elsevier Saunders. **6**, 117-129.
2. Alexandre S. Almeida, Fiske Christina T., Sterling Timothy R. & Kalamsa Spyros A. (2011). Increased Frequency of Regulatory T Cells and T Lymphocyte Activation in Persons with Previously Treated Extrapulmonary Tuberculosis. *Clinical and Vaccine Immunology,* **19**, 45-52.
3. Angela M. Green, DiFazio Robert & Flynn JoAnne L. (2012). IFN-g from CD4 T Cells Is Essential for Host Survival and Enhances CD8 T Cell Function during Mycobacterium tuberculosis Infection. *The Journal of Immunology,* **190,** 270-277.
4. Francesco Dieli, Friscia Giuseppe, Sano Caterina Di, Ivanyi Juraj, Singh Mahavir, Spallek Ralph, Sireci Guido, Titone Lucina & Salerno Alfredo (1999). Sequestration of T Lymphocytes to Body Fluids in Tuberculosis: Reversal of Anergy following Chemotherapy. *The Journal of Infectious Diseases,* **180,** 225-228.
5. Glaziou Philippe, Sismanidis Charalambos, Floyd Katherine & Raviglione Mario (2014). Global Epidemiology of Tuberculosis. *Cold Spring Harbor Perspectives in Medicine***,** 1-17.
6. Indonesian Health Ministry, (2014). Profile of Indonesian Health 2013. Jakarta, Indonesia.
7. Mensah Gloria Ivy, Addo Kennedy Kwasi, Tetteh John Amissah, Sowah Sandra, Loescher Thomas, Geldmacher Christof & Jackson-Sillah Dolly (2014). Cytokine response to selected MTB antigens in Ghanaian TB patients, before and at 2 weeks of anti-TB therapy is characterized by high expression of IFN-γ and Granzyme B and inter-individual variation. *BioMed Central Infectious Diseases Journals,* **14,** 1-11.
8. Mitchison D. A. (1993). Assessment of new sterilizing drugs for treating pulmonary tuberculosis by culture at 2 months. *The American. Review of Respiratory Disease Journals,* **147,** 1062-1063.
9. Nadia Caccamo, Guggino Giuliana, Meraviglia Serena, Gelsomino Giuseppe, Carlo Paola Di, Titone Lucina, Bocchino Marialuisa, Galati Domenico, Matarese Alessandro, Nouta Jan, Klein Michel R., Salerno Alfredo, Sanduzzi Alessandro, Dieli Francesco & Ottenhoff Tom H. M. (2009). Analysis of Mycobacterium tuberculosis-Specific CD8 TCells in Patients with Active Tuberculosis and in Individuals with Latent Infection. *PLoS ONE,* **4,** 1-10.
10. Pedro Godoy, A Nogues & M Alseda (2001). Risk factors associated to tuberculosis patients with positive sputum microscopy. *Gaceta Sanitaria Journals,* **15,** 506-512.
11. Prezzemolo Teresa, Guggino Giuliana, Manna Marco Pio La, Liberto Diana Di, Dieli Francesco & Caccamo Nadia (2014). Functional signatures of human CD4 and CD8T cell responses to Mycobacterium tuberculosis. *Frontiers in Immunology,* **5,** 1-13.
12. Raja Alamelu (2004). Immunology of Tuberculosis. *Indian Journal of Medical Research* **120,** 213-232.
13. Siri Laura Feruglio, Tonby Kristian, Kvale Dag & Riise Anne Ma Dyrhol (2014). Early dynamics of T helper cell cytokines and T regulatory cells in response to treatment of active Mycobacterium tuberculosis infection. *Clinical and Experimental Immunology***,** 1-29.
14. Sud Dhruv, Bigbee Carolyn, Flynn JoAnne L. & Kirschner Denise E. (2006). Contribution of CD8+ T Cells to Control of Mycobacterium tuberculosis Infection. *The Journal of Immunology***,** 4296-4314.
15. Veenstraa Hanne, Crous Ilse, Brahmbhatta Shweta, Lukey Pauline, Beyers Nulda, Heldena Paul D. van & Walzl. Gerhard (2007). Changes in the kinetics of intracellular IFN-γ production in TB patients during treatment. *Clinical Immunology,* 124**,** 336-344.
16. WHO 2014. Global Tuberculosis Report 2014. France: WHO Press.