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IFN-γ and IL-2 Secretion after ESAT-6-CFP-10 (EC-610) Fusion Antigen Stimulation from Patients with Active Lung Tuberculosis and Latent Lung Tuberculosis

Bastian[1*], Nova Kurniati[2], Kemas Ya'kub Rahadiyanto[3], M. Irsan Saleh[4], Zen Hafy[5], Francisca Srioetami Tanoerahardjo[6], Jusak Nugraha[7], Eddy Mart Salim[2]

[1] Department of Biomedical, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

[2] Division of Allergy and Clinical Immunology, Department of Internal Medicine, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

[3]Department of Clinical Pathology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

[4]Department of Pharmacology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

[5] Department Histology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

[6]Molecular and Microbiology Laboratory, TB Research Center for Biomedical and Basic Health Technology, National Center for Health Research and Development, Ministry of Health Republic of Indonesia, Jakarta

[7]Division Immunology, Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author email: bastian.darwin51@yahoo.com

Abstract

The Secretion of IFN-γ and IL-2 After ESAT-6-CFP-10 Fusion Antigen Stimulation in Active and Latent TB Patients. This study held to discover how immune responses work and to know the pathogenesis of active TB and latent TB patients. This study used PBMC to stimulate T Cells with ESAT-6 and CFP-10 antigen fusion, and measure the level of IFN-γ and IL-2 with ELISA antibody sandwich (U-Cytech). 16 ml of blood were drawn to 5 tubes. ESAT-6 CFP-20 inducted one tube with QuantiFERON for IFN-γ assay. The other four tubes were PBMC isolated using Ficoll-Paque, and pre-incubated with stimulation of ESAT-6 CFP-10 fusion antigen for 24-72 hours at 370 C and measured using T-Spot and ELISA reader. We got from this study that there are no significant differences in IFN-γ levels for both groups with active TB and latent TB. Measurement of IL-2 levels showed significant differences between the two group.

Keywords. tuberculosis, active TB, latent TB, Elisa antibody.

Introduction

Pulmonary tuberculosis is a chronic and specific infectious disease that can be transmitted directly, usually caused by the bacterium Mycobacterium tuberculosis, which is a microorganism that has infected a third of the world's population where the lung becomes infected target organs.[1] TB incidence in Indonesia (410 - 520 thousand), the latest data shows that Indonesia occupies the second warning of new TB cases in the world. In 2015 there were an estimated 10.4 million new tuberculosis or 142 cases / 100,000 population, with 480,000 multidrug-resistant cases.The results of Riskesdas conducted in 2013 showed the prevalence of TB based on a diagnosis of 0.4% of the population. Every 100,000 people in Indonesia, there are 400 people diagnosed with TB cases by health workers. Riskesdas 2013 is no different from Riskesdas 2007, where the prevalence of pulmonary TB is 0.4%.[2,3]

Individuals infected with TB germs, about 10% will develop active TB, and the remaining 90% become latent TB, which is characterized by an immune response against bacteria characterized by a positive IGRA examination, without active clinical infection, microbiologically or radiology shows negative results.

Scientists suspect immune system disorders in people with TB, where helper-1 (Th1) T cells play an essential role in the body's defense system, especially in dealing with intracellular bacterial infections.92. Shifting the balance between the production of cytokines from Th1 and Th2 cells can determine disease progression. According to Mortaz (2012), Th1 produces TNFα, IL-2, IFN-γ, cytokines from Th1 stimulate macrophages and cell-mediated reactions (CMR), which are critical in defense against intracellular pathogen infections, cytotoxic reactions and delayed-type hypersensitivity (DTH).[4]

The protective role of IFN-γ in tuberculosis is well known, especially in specific T cell immunity antigens. Production of IFN-specific mycobacterium antigens in vitro can be used as a marker of TB infection. Interleukin 2 is a specific cytokine produced by Th1, IL-2, which is released, will stimulate the activation and proliferation of other Th lymphocytes so that it gives a better response against antigens. Besides, IL-2 also increases proliferation, differentiation and enhance the cytolytic function of other immune cells namely NK cells and B cells which also play a role against MTB.[5]

Immunological examination now used in the diagnosis of TB patients is by the IGRA method and by the T-SPOT method. Commercially available IGRA: QuantiFERON-TB Gold in

- Tube assay (QFT-GIT), detect levels of IFN-γ produced in response to ESAT-6 and CFP-10 antigens, using the ELISA method. This is an indirect measurement of M. tuberculosis's specific cell, whereas the T-SPOT TB assay measures the number of cells producing IFN-γ in the M. tuberculosis antigen response ESAT-6, and CFP-10, and this is based on an enzyme-linked immune-sorbent spot (ELISPOT) examination.[6]

This research will use PBMC to stimulate T cells with ESAT-6-CFP-10 (EC610) fusion antigen, which will then see IFN-γ and IL-2 levels will be measured using the ELISA Antibody Sandwich using U-Cytech. The use of PBMC has not been done much, especially in Indonesia, and by knowing the levels of IFN-IL and IL-2 can find out how the immune response or, in other words, this research will compile patients' pathogenesis with active TB or latent TB.

Methods

This type of research is quasi-experimental. This method is used to determine the differences in IFN-γ and IL-2 secretion after ESAT-6-CFP-10 (EC610) fusion antigen stimulation from patients with active pulmonary tuberculosis and patients with latent pulmonary tuberculosis. With a design sample post-test only design. Sampling was carried out at the Palembang City Lung Special Hospital, and the examination was carried out at the Palembang City Lung Special Hospital Laboratory, which was carried out in the span time in August 2018-January 2019.

Venous blood sampling is carried out as much as 16 ml, inserted into a tube with a heparin anticoagulant. Subsequently, part of one of the tubes was carried out Induction with ESAT-6-CFP-10 protein with the QuantiFERON method for IFN-γ examination that was incubated for 16-24 hours at 370C and the other part of the sample or four tubes was carried out by PBMC using Ficoll-Paque, then induced with ESAT-6-CFP-10 Fusion Antigen and incubated for 24 -72 hours at 370C. Next, IFN-γ and IL-2 levels were measured by T-Spot and ELISA-Reader.

Results

Examination data that has been obtained, then carried out statistical data analysis, which then obtained the following results:

Table 1. Levels of IFN-γ in sufferers of active TB and latent TB

IFN-γ	Clinical status	n	Median (Min-Max)	*p*
Antigen	Active TB	21	6700 (2000-9100)	0,769*
EC610 Fusion	Latent TB	28	6000 (2500- 9000)	

* *Mann-Whitney test,* p<0.05. With the p value obtained = 0.769

The test results showed that there were no significant differences between IFN-γ levels in the active or latent TB groups after stimulation of EC610 Fusion Antigen, which is indicated by p > 0.05.

The data that has been obtained is then analyzed statistically, which then obtains the following results:

Table 2. IL-2 levels in patients with active TB and latent TB

IL-2	Clinical Status	N	Mean (SD)	*p*
Antigen	Active TB	21	2309.52 (560.272)	0,013*
Fusion EC610	Latent TB	28	1903.57 (530.187)	

**Independent T-Test, p<0.05*

The results of the table show that there are significant differences between UL-2 levels in the Active TB and latent TB groups after stimulation with the EC610 Fusion Antigen, which is indicated by p <0.05.

Discussion

The immune response to Mycobacterium tuberculosis in addition to the protective Th-1 cell immune response also stimulates the immune response of the allegedly non-protective Th-2 cell. Macrophage cells release IL-12, inducing T naïve (Th-0) cells to differentiate into Th-1 cells and Th-2 cells. Furthermore, IL-1 from macrophages induces Th-1 cells to produce cytokines IFN-γ, IL-2 and TNF-α. IFN-γ and IL-2 induce Th-2 cells to produce cytokines IL-4, IL-5, IL-6 and IL-10 (Sudiana, IK. 2014). Furthermore, IL-4, IL-5 and IL-6 induce B lymphocytes to be differentiated into memory cells and plasma cells to produce antibodies (Ab).[7-9]

The results showed that the mean level of IFN-γ after stimulation with EC-610 antigens in patients with active TB was higher than even in latent TB although there was no statistically significant difference (p = 0.769) between the two sample groups. This study is in line with Setiawan and Nugraha (2010) which states that on average IFN-γ levels are higher in active TB compared to latent TB stimulated with ESAT-6 and CFP-10 although there is no statistically significant difference.[10]

Higher levels of INF-γ in active TB are obtained because there is a protective immune response to TB germ infection. 78. Another study found the same results as this study, regarding IFN-γ levels in active pulmonary TB patients compared with healthy people. The study found that the average IFN-γ level in healthy control subjects was lower than in TB patients. This may be because most active TB patients are diagnosed as being on anti-tuberculosis therapy, and therefore the healing effect on granulomas can increase the number of T cell production which will increase IFN-γ levels.[11,12]

Some recent research also shows that the process of autophagy plays an essential role at the end of M. tuberculosis infection. Induction of autophagy will mediate antimicrobial activity. In the adaptive immune response, autophagosomes will cause the intracellular substrate to move, including pathogens that invade macrophages into MHC class II complexes for stimulation of CD4 + T cells. Autophagy can be induced by most cytokines produced by polarized Th1, such as IFN-γ and TNF-α, and are needed for host defence against M. tuberculosis.[13]

The percentage of T-CD4+ cells expressing IFN-γ is higher in active pulmonary TB patients compared to latent TB. The mean T-CD4+ cells that express IFN-γ are higher in active pulmonary TB patients, indicating that although IFN-γ is needed to protect, IFN-γ alone is not enough for that of M. Tb or maybe the level of IFN-γ produced by T-CD4 + cells is still not

enough to fight the growth of M. tuberculosis. The difference in the percentage of existing T-CD4 + cells expressing IFN-γ between active and latent pulmonary TB patients may be caused by in active-pulmonary TB there is an emphasis on the activity of T-CD4+ cell function by Treg cells or by monocyte/macrophage / DC yields such as TGF-β or IL-10, i.e. those not examined in this study; The non-prominent role of T-CD4+ cells in latent TB in producing IFN-γ compared to in active- pulmonary TB.[14-16]

In populations infected with TB naturally in endemic areas, it shows that IFN-γ levels are significantly higher in TB patients. This shows that ESAT-6 / CFP-10 is not related to protective immunity associated with T cell, as measured by IFN-γ production, as previously known. These results indicate that ESAT-6/CFP-10 is associated with clinical TB or disease progression. The ESAT-6/CFP10 complex has been reported to be able to bind to monocyte cells through the C-terminal flexible arm of CFP-10.2 In another study, and it was reported that ESAT-6 binds to T cells, monocytes and B cells, whereas CFP-10 binds only to monocytes and B cells, but does not reduce viability or increase apoptosis of these cells or does not inhibit IL-2 production.[17]

This study found that IL-2 levels in TB patients were 2309.52 pg/mL and in latent TB patients was 1903.57 pg/mL with a p-value = 0.013. The results show that there is a significant difference where IL-2 levels in active TB sufferers are higher than latent TB sufferers. The results of this study are consistent with the theory that IL-2 increases occur depending on whether or not the stimulation provided by APC to naive lymphocyte cells. In TB infection, M.tb as an intracellular pathogen is presented through MHC class 1 by APC, which then activates CD8 T cells. This will then increase the cytokines that play a role in the maturation and proliferation of CD8 T cells such as IL-2.

M.tb specific antigen strongly influences the formation of IL-2 in LTBI patients where after 18 hours of incubation, it is found that IL-2 levels are higher in active TB compared to latent TB however, when the incubation time was extended to 72 hours, the amount of IL-2 secreted by patients with latent TB was significantly higher than that secreted by active TB patients, These results indicate that IL-2 levels are affected by prolonged incubation or antigen exposure which will cause an immune response.[18]

According to Millington et al. (2011) an increase in the amount of IL-2 secretion and memory central T cell that secretes IL-2 and a reduced number of effector memory T cells that secrete IFN-γ in patients with latent TB infection, in comparison with active TB people, which

shows the predominance of IFN-γ secretion cells during the active TB phase. Increased levels of IL-2 in people after undergoing anti-tuberculosis treatment, maybe due to expansion of central memory T cell, by decreasing the amount of M. tuberculosis antigen, which is also present in latent TB patients.

IL-2 response is higher compared to latent TB due to an increase in the number of central memory T cells in active-TB. Given that the positive ELISPOT IL-2 results are associated with prolonged exposure, an increase in memory cells that secrete IL-2 might explain the high IL-2 response that is in line with the increase in IFN-γ levels in this study.[19]

An increase in IL-2 secretion in the treatment phase is due to an increase in specific TB cells that produce IL-2 during treatment. Evaluation of the combination of cytokine markers can be an alternative way to monitor treatment response, compared with only one cytokine response. Repeated measurements of IFN-γ and IL-2 levels help determine the profile of T cell cytokines, which display memory phenotypes and include three main functions of T cell subsets: effector cells that secrete IFN-γ only, memory effector cells that secrete IFN-γ and IL-2, and central memory cells that only secrete IL-2.[19-20]

The proportion of cell-specific ESAT-6 / CFP-10 that secretes only IFN-γ decreases during treatment. In contrast, the proportion of ESAT-6-CFP-10 cells, specifically secreting IFN-γ / IL-2, increases. The increase in IL-2 / IFN-γ comparison in this study can be explained by the presence of specific TB cell dynamics.[7,19]

Conclusion

The levels of IFN-γ after stimulation of ESAT-6-CFP-10 (EC610) Fusion Antigen in active-TB and latent TB were not significantly different between the two groups while IL-2 levels after ESAT-6- CFP-10 (EC610) Fusion Antigen stimulation on active TB and latent TB showed that there were significant differences between the two groups.

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