

# INTERFERON GAMMA LEVELS AS EX-VIVO IMMUNOGENICITY TEST OF NEW TUBERCULOSIS VACCINE CANDIDATE: ESAT-6-AG85C-POLYHISTAG ANTIGEN FUSION

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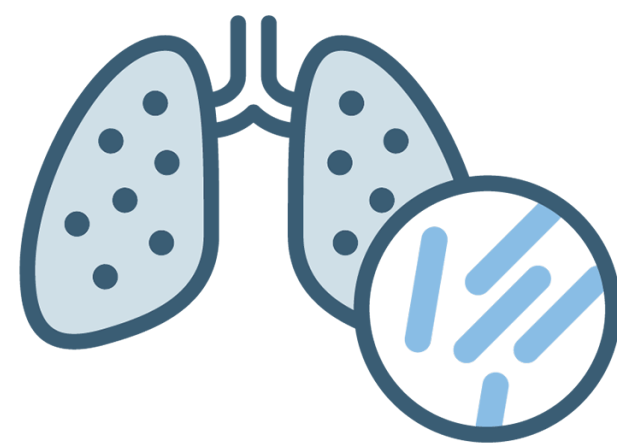
## 1 Background



The effectiveness of the BCG vaccine in adults varies greatly, and its protective effect is not significant after 10 years. Recombinant vaccine consisting of two immunodominant Mtb antigens ESAT-6-Ag85C-polyHistag (EAH) is being developed as new TB vaccine candidate for booster. Immunogenicity testing by measuring IFN $\gamma$  is required to evaluate the immune response to this TB vaccine candidates.

## 2 Method

Seventeen newly diagnosed active pulmonary TB patients and 16 TB patients in treatment >2 months were recruited from March 2018 to January 2019 in Balai Besar Kesehatan Paru Masyarakat Bandung and Dr. Hasan Sadikin Hospital Bandung.



TB diagnosis were performed according to WHO criteria, on the basis of clinical presentation and chest X-ray, confirmed by microscopic detection of acid fast bacilli in Ziehl-Neelsen stained sputum smears or positive Mtb detection on Expert MTB/Rif. HIV positive, HBsAg positive, anti HCV positive and diabetes patients were excluded. Ten healthy individuals were included as control subjects.

Peripheral blood mononuclear cells were obtained from 8ml of heparinized blood in BD Vacutainer<sup>®</sup>CPT that processed within 2 hours after blood collection. TB EAH antigen (50  $\mu$ g/mL) were added to PBMC and incubated at 37°C, supernatant were harvested after 48 hours and stored at -20°C until IFN $\gamma$  measured. IFN $\gamma$  were measured with enzyme-linked immunosorbent assay according to manufacturer's protocol.

## 3 Result

A total of 57 subjects were recruited in this study and 14 of them were excluded from this study. Baseline characteristics of the subjects are presented in Table 1. There was no significant difference in median age, leukocyte value, and PBMC count between 3 groups.

The highest median of IFN $\gamma$  after stimulation with TB EAH antigen was found in new case pulmonary TB group. There were significant differences in the median IFN $\gamma$  levels among three groups as illustrated in Table 2.

### References

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Table 1. Baseline Characteristics

| Characteristics                                      | New Case Pulmonary TB (n=16) | Pulmonary TB on Treatment (n=17) | Healthy Individuals (n=10) |
|------------------------------------------------------|------------------------------|----------------------------------|----------------------------|
| <b>Sex</b>                                           |                              |                                  |                            |
| Male; n(%)                                           | 9 (56)                       | 12 (71)                          | 5 (50)                     |
| Female; n(%)                                         | 7 (44)                       | 5 (29)                           | 5 (50)                     |
| <b>Age(years); median (min-max)</b>                  | 31 (18-65)                   | 26 (18-48)                       | 33 (24-46)                 |
| <b>Laboratory</b>                                    |                              |                                  |                            |
| Leukocyte (/mm <sup>3</sup> ); median (min-max)      | 7,840 (4,310-14,920)         | 7,440 (4,240-9,800)              | 7,035 (4,610-11,950)       |
| PBMC count/well (10 <sup>3</sup> ); median (min-max) | 5,080 (1,250-17,150)         | 6,100 (2,550-11,800)             | 8,850 (2,760-10,540)       |

Table 2. IFN $\gamma$  levels in New Case Pulmonary TB, Pulmonary TB on Treatment and Healthy Individuals after TB EAH Antigen Fusion Stimulation

| IFN $\gamma$ (pg/mL)    | Groups                |                           |                     | p value* |
|-------------------------|-----------------------|---------------------------|---------------------|----------|
|                         | New Case Pulmonary TB | Pulmonary TB on Treatment | Healthy Individuals |          |
| <b>Median (min-max)</b> | 17.09 (2.66-140.14)   | 4.36 (2.43-21.41)         | 2.91 (2.39-3.86)    | 0.001    |

Post hoc analysis to test IFN $\gamma$  levels differences after TB EAH stimulation between 2 groups shows significant differences between all the groups, with the highest statistical significance is between new case pulmonary TB and healthy individuals (p=0.001) followed by the new pulmonary TB groups compared to the pulmonary TB in treatment >2 months group (p = 0.012) and between the pulmonary TB in treatment >2 months compared to healthy people (p=0.035).

High IFN $\gamma$  levels in new case pulmonary TB reflects the activity of the disease. It has been suggested that lower IFN $\gamma$  production after treatment of active TB indicates successful treatment that reduce bacterial load and the number of local and circulating IFN $\gamma$  producing activated T cells. The limitation of this study was functional T cell assay was not performed, so other variables that can influence T cell function cannot be analyzed.

## 4 Conclusion



In conclusion, there were significant differences of IFN $\gamma$  levels between new case pulmonary TB, pulmonary TB on treatment and healthy individual group after ESAT6-Ag85C-polyHistag antigen fusion stimulation. This result suggest that this immunodominant antigen fusion is potential for tuberculosis vaccine development.

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