RINGKASAN

Sekresi Interleukin 12 pada Kultur Makrofag dari Penderita Tuberkulosis Paru dan Individu Sehat Berisiko Tuberkulosis Paru, yang masing-masing diinfeksi *Mycobacterium tuberculosis*

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Tuberkulosis (TB) adalah penyakit infeksi kronis disebabkan oleh *Mycobacterium tuberculosis* yang terutama menyerang jaringan paru. Sejak 1985 sampai saat ini tuberkulosis telah menjadi masalah kesehatan dunia. Patogenesis tuberkulosis ditentukan interaksi antara imunitas hospes dengan *Mycobacterium tuberculosis*, *activated* makrofag berusaha mengeliminasi *Mycobacterium tuberculosis* namun *Mycobacterium tuberculosis* memiliki mekanisme *evasion* sehingga mampu bertahan dan bermultiplikasi.

Interleukin 12 merupakan salah satu kunci pertahanan imunitas melawan *Mycobacterium tuberculosis*, disekresikan *activated* makrofag, berfungsi menginduksi sekresi IFN-γ oleh sel NK dan sel T, serta merangsang differensiasi Th0 menjadi Th1. IFN-γ meningkatkan kemampuan mikrobisidal makrofag sehingga mampu membunuh *Mycobacterium tuberculosis*.

Tujuan penelitian adalah mengetahui sekresi Interleukin 12 antara kelompok kultur makrofag dari penderita tuberkulosis paru dengan kelompok kultur makrofag dari individu sehat berisiko tuberkulosis paru setelah diinfeksi *Mycobacterium tuberculosis* dengan inkubasi selama 24 jam dan 48 jam.

Penelitian ini dilakukan dengan isolasi Peripheral Blood Mononuclear Cells (PBMC) dari *buffy coat* darah vena cubiti individu sehat berisiko tuberkulosis paru dan penderita tuberkulosis paru. Sel-sel diletakkan diatas coverslip dalam sumuran 24-well, dengan konsentrasi $2x10^5$ sel per sumuran. Sel PBMC tersebut kemudian dikultur dalam inkubator CO2 pada 37^0C, 5% CO2 dengan suplementasi RPMI, 10% *pooled human serum* dan 100 iu/ml penicillin. Pada hari ke 2 dilakukan pencucian dengan RPMI 5 kali untuk membuang sel-sel limfosit. Kultur dilanjutkan dan setiap hari dilakukan penggantian medium, kemudian hari ke 4 setelah sel-sel monosit berdiferensiasi menjadi makrofag lalu di infeksikan dengan *Mycobacterium tuberculosis* 1,5 x 10^5 sel/sumuran, setelah diinfeksi diperiksa sekresi interleukin 12 pada supernatan kultur makrofag pada inkubasi selama 24 jam dan 48 jam.

Analisis statistik dilakukan dengan menggunakan ANOVA (*Analysis of Variance*) yang digunakan untuk membandingkan hasil-hasil yang diperoleh pada kelompok perlakuan yang berbeda, dan hasil yang bermakna dilanjutkan dengan uji LSD (*Least Square Difference*).

Hasil penelitian menunjukkan sekresi Interleukin 12 pada kultur makrofag individu sehat berisiko tuberkulosis paru setelah 24 jam diinfeksi *Mycobacterium tuberculosis* (3,156 ng/ml) lebih tinggi dibandingkan pada penderita tuberkulosis paru (1,593 ng/ml) dan sekresi IL-12 pada kultur makrofag individu sehat berisiko tuberkulosis paru setelah 48 jam diinfeksi *Mycobacterium tuberculosis* (3,446 ng/ml) lebih tinggi dibandingkan pada penderita tuberkulosis paru (1,8 ng/ml). Hasil analisis ANOVA pada waktu inkubasi 24 jam dam 48 jam setelah infeksi menunjukkan perbedaan yang sangat bermakna p=0,000.

Kesimpulan dari penelitian ini adalah sekresi Interleukin 12 kelompok kultur makrofag individu sehat berisiko tuberkulosis paru yang diinfeksi *Mycobacterium tuberculosis* pada inkubasi 24 jam dan 48 jam lebih tinggi dibandingkan pada kelompok kultur makrofag dari penderita tuberkulosis paru.

Perlu dilakukan studi *invitro* untuk mengetahui efek Interleukin 12 pada komponen sistem imun lainnya seperti pada kultur limfosit untuk mengetahui efek Interleukin 12 terhadap sekresi IFN-γ, perlu dilakukan penelitian *invivo* untuk mengetahui apakah Interleukin 12 bisa berperan sebagai imunomodulator yang nantinya bisa digunakan untuk imunoterapi terutama dalam pengobatan tuberkulosis dan perlu penelitian lebih lanjut mengenai adanya defek gen penyandi interleukin 12 pada penderita tuberkulosis paru.

SUMMARY

Interleukin 12 Secretion in The Culture of Macrophage from Pulmonary Tuberculosis Patients and Healthy Individuals with Pulmonary Tuberculosis Risk, that infected with *Mycobacterium tuberculosis*

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Tuberculosis is a chronic infectious disease caused by *Mycobacterium tuberculosis* which mostly attacks pulmonary tissue. Since 1985 until now, tuberculosis have been a global health problem.

Pathogenesis of tuberculosis is mainly determined by interaction between host immunity and *Mycobacterium tuberculosis*. Activated macrophages try to eliminate *Mycobacterium tuberculosis,* but on the other hand, *Mycobacterium tuberculosis* has evasion mechanism from host immunity, so that it can survive and multiplicate.

Interleukin 12 is a crucial cytokine which acts as one of key defence immunity from tuberculosis infection and has the following main functions : induces IFN-γ production by T cells and Natural killer (NK) cells, induces differentiation from Th0 becomes Th1. IFN-γ would influence macrophage to be more active so that it can increase microbicidal activity to kill *Mycobacterium tuberculosis*.

The purpose of this study was to find whether there was any difference in interleukin 12 secretion capacity between macrophages culture from pulmonary tuberculosis patients and healthy individuals with pulmonary tuberculosis risk after being infected with *Mycobacterium tuberculosis* at incubation period of 24 hours and 48 hours.

The experiment was performed by isolation of *Peripheral Blood Mononuclear Cells* (PBMC) from buffy coat of pulmonary tuberculosis patients and healthy individuals with pulmonary tuberculosis risk. The cells were plated on coverslip glass in 24-well tissue culture plates with concentration of $2x10^5$ cells/well and cultured at CO2 incubator in RPMI supplemented with 10% pooled human serum and 100 iu/ml penicillin at 37^0C; 5% CO2. On day II, wells were washed 5 times with RPMI to eliminate lymphocytes and then after feeding, the culture were continued. On day IV, culture were infected with 1,5 x 10^5 *Mycobacterium tuberculosis* for each well. After

infection, Interleukin 12 secretion was measured at incubation period of 24 hours and 48 hours with ELISA (Accucyte human IL-12) procedure.

Analysis of Variance (ANOVA) was used to compare the result obtained with different treatment and Least Square Difference (LSD) was used to continue analysis for significant result.

Result showed Interleukin 12 secretion at macrophage culture of healthy individual with pulmonary tuberculosis risk at incubation period of 24 hours (3,156 mg/ml) were more higher than pulmonary tuberculosis patients (1,543 ng/ml) and Interleukin 12 secretion at macrophage culture of healthy individual with pulmonary tuberculosis risk at incubation period of 48 hours (3,446 mg/ml) were more higher than pulmonary tuberculosis patients (1,543 ng/ml). ANOVA showed significant difference (p=0,000) in interleukin 12 secretion between macrophage culture of pulmonary tuberculosis patients and healthy individuals with pulmonary tuberculosis risk after *Mycobacterium tuberculosis* infection at incubation period of 24 hours and 48 hours.

In conclusion, there was a difference in the secretion of interleukin 12 between that in the macrophage of pulmonary tuberculosis patients and that in the macrophage of healty individual with pulmonary tuberculosis risk, that infected with *Mycobacterium tuberculosis* at incubation period of 24 hours and 48 hours.

An invitro study is needed to find the effect of interleukin 12 on other immune system components for example, in lymphocyte culture the study should be aimed to find the effect on IFN-γ secretion, In vivo study is also needed to determine whether interleukin 12 may act as immune modulator that can be applied for immuno therapy particularly in tuberculosis treatment and study to determine Interleukin 12 gen defect at pulmonary tuberculosis patients.

ABSTRACT

Interleukin 12 in The Culture of Macrophage from Pulmonary Tuberculosis Patients and Healthy Individuals with Pulmonary Tuberculosis Risk, that Infected with *Mycobacterium tuberculosis*

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This study was to evaluate Interleukin 12 secretion at macrophage culture of pulmonary tuberculosis patients and healthy individuals with pulmonary tuberculosis risk who was infected with *Mycobacterim tuberculosis*.

Activated macrophage is the major phagocytic cells involved in protection against *Mycobacterium tuberculosis* infection. Interleukin 12 is secreted by activated macrophage.It plays an important role in IFN-γ production by T cells and NK cells and also influences the differentiation of Th0 to become Th1. IFN-γ induced bacterisidal mechanism in activated macrophage, so that it can kill *Mycobacterium tuberculosis*.

PBMC was isolated from pulmonary tuberculosis patients and healthy individuals with pulmonary tuberculosis risk. PBMC were cultured for 24 hours and then washed with RPMI 5 times to clear the culture from lymphocytes. The culture was maintained at CO2 incubator; at 37^0C; 5% CO2 and every day the feeding is carried out until day IV when the cells were infected with *Mycobacterium tuberculosis*. After infection, interleukin 12 secretion was measured by ELISA at incubation period of 24 hours and 48 hours.

Result showed Interleukin 12 secretion at macrophage culture of healthy individual with pulmonary tuberculosis risk at incubation period of 24 hours and 48 hours were more higher than pulmonary tuberculosis patients. ANOVA showed significant difference (p=0,000) in interleukin 12 secretion between macrophage culture of pulmonary tuberculosis patients and healthy individuals with pulmonary tuberculosis risk after *Mycobacterium tuberculosis* infection at incubation period of 24 hours and 48 hours.

Key word: *Mycobacterium tuberculosis*, activated macrophage, Interleukin 12.