Original Article

Cytokines Dynamics in a Wistar Rat Model Infected with *Mycobacterium Tuberculosis* Strain H37Rv

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Abstract

Background: The immunology characteristics of the rat model of tuberculosis (TB) infection are still unclear. This study aimed to evaluate the dynamics of pathology and cytokines in a rat model infected with *Mycobacterium* TB (MTB). **Methods:** Sixty male Wistar rats were divided into four groups, namely the control group (without MTB infection) and the MTB -induced group (observations at week-3, week-6, and week-12 postinfection). Granuloma formation was analyzed by histology procedure. Analysis of the levels of tumor necrosis factor-a (TNF-α), interleukin-6 (IL-6), IL-17, IL-2, IL-4, and IL-12 was performed using an enzyme-linked immunosorbent assay technique. **Results:** The number and size of the ganulomas increased proportionally between weeks 6 and 12 postinfection. Several cytokines, namely IL-6, IL-17, IL-2, IL-4, and IL-12 significantly increased in the 6th week compared to the 3rd week after infection ($P < 0.05$). These cytokines decreased significantly at the 12th week compared to the 6th week ($P < 0.05$). TNF-α was found to be stable at the third and 6th weeks and then decreased at the twelfth postinfection week. For IL-12, the longer the infection time, the higher the level. **Conclusions:** It was concluded that there was a typical pattern of TB infection in Wistar rats, namely certain cytokines that peaked at week 6 of infection. Thus, TB infection in rats can be a model for early-phase TB study.

Keywords: Experimental animal models, granuloma, immunology, pathology, pulmonary tuberculosis

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Introduction

Tuberculosis (TB) is a granulomatous infection caused by the bacteria *Mycobacterium* TB (MTB). Southeast Asia has the highest incidence of TB, accounting for nearly 40% of all cases worldwide. Each year, Southeast Asian countries, namely India, Bangladesh, Thailand, Myanmar, and our country Indonesia, generate 3.4 million new cases.[1] With high cases (including the top five diseases in Indonesia),[2,3] treatment resistance,[4,5] and attacks on the adolescent population,[6,7] the TB situation in Indonesia is particularly complex. Furthermore, Indonesia, India, and Nigeria account for half of all unrecorded or undiagnosed cases,[8,9] particularly latent TB.[9,10]

Aside from the concerns mentioned above, one of the ongoing causes of TB is the lack of an appropriate animal model. Rats, mice, guinea pigs, and rabbits are used as TB model animals. Mice are not sensitive to tuberculin. Guinea pigs transmit illnesses similar to those found in newborns and sick people. The rabbit is a TB animal model that closely reflects human disease characteristics.[11] These various animal models were chosen for their economy, infection mechanism, and application goal.[12] However, the use of animal models for TB studies is currently being debated. The goal of this study is to look at the histology profile and cytokine levels in a MTB -infected rat.

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Methods

Animals

Male Wistar strains weighing 200–250 g were used in this study. These rats were collected from the animal house of the

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Bogor Veterinary Research Institute in West Java, Indonesia. Rats were housed in biosafety level 3 cages at the Veterinary Research Institute in Bogor, West Java, Indonesia. The rats were acclimatized for 7 days before to the experiment. During the trial, the rats were fed regular chow and had unfettered access to water.

Tuberculosis rat model

MTB strain H37Rv from the American Type Culture Collection was utilized to create this disease model. In order to cause pulmonary TB, an inhalation approach was used. MTB was breathed using aerosolids for 30 min. The bacterial concentration per lung was 10^5 colony forming units. Three weeks after the infection, the first examination was performed. The second and third observations were made 6 and 12 weeks after infection.

Blood sampling

Ketamine was administered intramuscularly into the rat thigh muscle for surgery. Blood samples were drawn from the posterior vena cava after thoracic surgery. While the heart was still beating, blood was drawn. The blood was then placed in a vacutainer to separate the serum. For 15 min, blood samples were centrifuged at 3000 rpm. The serum was stored at −40°C overnight before being moved to −80°C the next day for testing. The lobes of the lung were all taken for histological study.

Cytokines analysis

An enzyme-linked immunosorbent assay (ELISA) was used to assess tumor necrosis factor (TNF)-and interleukin (IL) such as IL-6, IL-17, IL-2, IL-4, and IL-12 in serum. The analysis was carried out in accordance with the kit's detailed procedure. Rat IL-6 ELISA (Bioenzy, Jakarta, Indonesia, Catalog No BZ-08185310-EB), Rat IL-17 ELISA (Bioenzy, Jakarta, Indonesia, Catalog No BZ-08185110-EB), Rat IL-2 ELISA (Bioenzy, Jakarta, Indonesia, Catalog No BZ-08183210-EB), Rat IL-4 ELISA (Bioenzy, Jakarta, Indonesia, Catalog No BZ-08183310-EB), Rat Tumor Necrois Factor Alpha ELISA (Bioenzy, Jakarta, Indonesia, Catalog No BZ-08184670-EB), Rat IL-12 ELISA (Bioenzy, Jakarta, Indonesia, Catalog No BZ-08189041-EB), and Rat IL-10 ELISA (Bioenzy, Jakarta, Indonesia, Catalog No BZ-08188010-EB) were applied for those assay.

Results

Histological marker

Figure 1 depicts the histological alterations in the lung throughout the course of several weeks of monitoring. Nonspecific nongranulomatous pneumonia was observed in the 3rd week. Granulomas of various sizes began to emerge at 6 weeks. Multinucleated giant cells were found at week 12.

Cytokines marker

Table 1 shows the cytokine levels in various week of observation. TNF-α levels in all weeks of observation increased significantly compared to controls ($P < 0.5$). The mean TNF-α levels were comparable between the third and 6th weeks ($P > 0.05$), then decreased significantly at the 12th week compared to the 6th week ($P < 0.5$). For IL-6, IL-17, IL-2, IL-4, and IL-12 were found to be higher at all weeks of observation than controls ($P < 0.5$). The levels of IL-6, IL-17, IL-4, and IL-12 at the 6th week were significantly higher than the third and 12th weeks ($P < 0.5$). IL-2 levels at the 12th week were significantly higher than the 3rd and 6th weeks ($P < 0.5$).

Discussion

Pathology of animal model of tuberculosis

Our findings show the clinical and immunological characteristics of MTB infection induced by inhalation. Inhaling MTB caused logarithmic bacterial multiplication in experimental animals for up to 3 weeks, according to earlier research. Furthermore, it will enter a chronic phase with no increase in bacterial levels in the lungs.[13] Pathological results in this investigation revealed nonspecific nongranulomatous pneumonia (week 3), the presence of granulomas of various sizes (week 6), and multinucleated giant cells (week 2). Furthermore, the number and size of granulomas peaked after 6 weeks. Granuloma formation takes 2–6 weeks and is produced by an immune response that causes lymphocytes and macrophages to collect at the lesion site.[14] The histology findings of this investigation are consistent with a previous publication.[15]

Cytokines profile of animal model of tuberculosis

T cells' particular responses to MTB and cytokine expression differ based on infection status.[16] Cluster of differentiation 4 (CD4) and CD8 T lymphocytes help to protect against MTB infection. CD4 T cells produce interferon gamma (IFN-γ) and TNF-α through the action of $CD8^+$ T cells, as well as cytotoxicity against infected macrophages.[17,18] IFN-γ

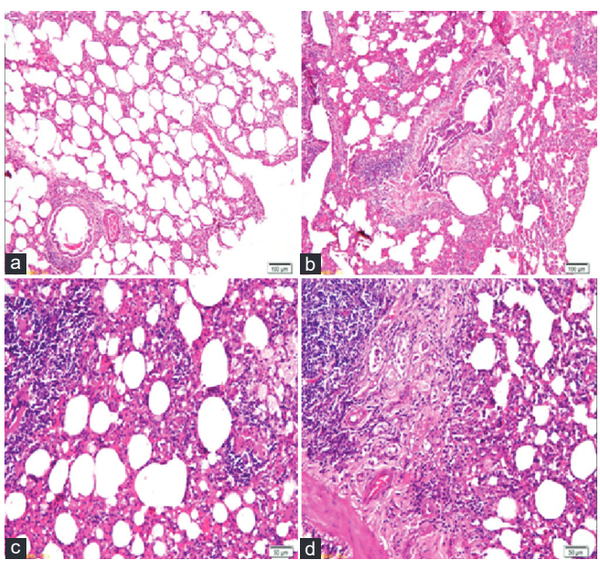

Figure 1: The histology of lung from all groups. (a) Control, (b) tuberculosis group at weeks 3 of observation, (c) tuberculosis group at weeks 6 of observation, (d) tuberculosis group at weeks 12 of observation

Table 1: The levels of cytokine in each weeks of observation

Cytokine	Control	Weeks 3	Control	Weeks 6	Control	Weeks 12
TNF-α	256.87±16.47	409.09±24.5[a]	307.81±21.53	436.08±15.61[a]	236.4±12.13	372.1±26.54[a,c]
IL-6	10.74±1.35	15.57±0.63[a]	11.4±0.90	17.62±0.53[a,b]	10.17±0.66	13.9±1.31[a,b,c]
IL-17	389.2±25.88	615.48±10.84[a]	463.97±30.75	664.43±5.07[a,b]	235.94±4.22	576.09±6.41[a,b,c]
IL-2	633.4±45.25	759.84±162.12[a]	671.09±35.50	893.04±32.18[a,b]	553.68±73.36	909.59±92.79[a,b,c]
IL-4	140.59±2.92	202.94±3.99[a]	142.56±2.38	210.48±1.38[a,b]	138.61±2.55	150.37±5.39[a,b,c]
IL-12	34.2±0.46	56.91±2.39[a]	33.89±2.53	60.23±1.11[a,b]	33.13±1.53	43.51±1.38[a,b,c]

[a]$P<0.05$ in comparison with its control group at similar observation time, [b]$P<0.05$ in comparison with infection group at weeks of 3, [c]$P<0.05$ in comparison with infection group at weeks of 6. IL: Interleukin, TNF-α: Tumor necrosis factor-α

and TNF-α are particularly important for macrophage antimicrobial activation.[15,19,20] In addition to these events, IL-17 is also involved in the control of primary infection.[21] Recent data demonstrate that IL-6 is not a predictor of elevated IL-17 in experimental animals infected with aerosolized MTB.[22]

In this study, we found a biphasic profile for the cytokines IL-6, IL-17, IL-4, and IL-12, with an increase at 6 weeks and a drop at 12 weeks. A linear pattern was discovered for TNF-α in the early weeks, which then reduced at week 12. The longer the observation, the higher the levels of IL-2. This finding suggests that IL-6, IL-17, IL-4, and IL-12 all have the same secretory profile. As a result of the interaction between MTB and macrophage receptors, IFN-γ, IL-2, IL-12, and TNF-α are produced as a signal of infection. This signal is designed to transport monocytes from the bloodstream to the affected lung.[23,24]

TNF-α levels were comparable at weeks 3 and 6, but then fell at week 12. Comparable levels for weeks 3 and 6 imply that TNF-α attempts to limit bacterial proliferation in organs in the early stages of infection and is present both at the site of infection and in the circulation for signaling.[23,25] High TNF-α levels aid in the spread of granulomas, the severity of infections, and the improvement of pathology.[26] A reduction in week 12 indicates that the signal in the circulation is fading.

Previous study has suggested that IL-6 is associated with sickness.[27] In this study, IL-6 levels were highest at week 6 compared to weeks 3 and 12. The increase from the third to the 6th week indicates that IL-6 acts as a proinflammatory cytokine. IL-6 is the only cytokine that increases in response to cell-mycobacterium interactions.[28] The decline at week 12 indicated that the bacterial load had stabilized and that there was no interaction between cells and bacteria.

In the presence of MTB infection, IL-2 is a cytokine generated by T cells that aids in cellular immunity. IL-2 is used in patients to distinguish between latent and active TB by enhancing the latent state.[29-31] Reduced bacterial replication and antigen are associated with an increase in secreting T cells.[32] We hypothesize that elevated IL-2 levels indicate TB latency, as evidenced by poor replication and antigen levels. In contrast, IL-2 is an inflammatory cytokine.[31] According to our findings, the increase at week 6 is due to its action as an inflammatory cytokine. A latency phase arises from an increase in week 12.

Collaboration between macrophages and T cells is necessary for TB defense. IL-12 is the major immune response cytokine.[33,34] In our study, IL-12 levels peaked at 6 weeks and then dropped at 12 weeks. This suggests that IL-12 was present at week 6 as a result of collaboration between macrophages and T cells and then decreased at week 12. IL-4 experienced the same fate. Because IL-4 can downregulate the immune response, it can lead to failure to treat sickness.[26]

Although it causes harm in advanced TB, IL-17 is useful in TB prevention by recruiting neutrophils.[35] Peak levels of IL-7 were reported at 6 weeks as opposed to 3 and 12 weeks in this investigation. In the 12th week, there was a decrease. This shows that IL-17 secretion is intended to prevent TB until the 6th week. Furthermore, IL-17 levels decrease as the organism enters a dormant condition. The revelation that activating H37RvMTB enhances IL-17 production in neutrophils, which is dependent on the paracrine activities of IL-6 and IL-23, backs up the findings in this rat model. The comparability of IL-6 and IL-17 profiles was confirmed in our study.[36]

Conclusions

It was concluded that there was a typical pattern of TB infection in mice, namely cytokines and granuloma pathology peaked at 6 weeks of infection. Thus, TB infection in mice can be a model for early-phase TB research.

Limitation of study

The limitation of this study is that chemokine measurements and cell population analysis were not carried out so as to provide a complete immunological profile.

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Ethical statement

All experimental animals were kept in facilities according to the protocols approved by the Research Ethics Committee

of the Faculty of Medicine, Sebelas Maret University, Surakarta, Central Java, Indonesia. (Number 758A/III/LPPM. PM.10.05/07/2020).

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Conflicts of interest

There are no conflicts of interest.

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