ORIGINAL PAPER

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Kinetics of Granuloma, IFN-γ and IP-10 in a Wistar Rat Model Infected with Mycobacterium Tuberculosis

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ABSTRACT

Background: Improved access and treatment are critical to controlling the problem. Molecular diagnostic tests, although available, are not feasible in developing countries. Where available, these tests require state-of-the-art laboratories and are not cheap. One alternative to molecular diagnostics is the use of acute phase protein values. This protein is a protein whose levels will increase or decrease in plasma in response to injury or inflammation Objective: This study aimed to analyze the kinetics of granulomas and the role of IFN-γ and IP-10 in the pathology of tuberculosis in a rat model. Methods: Sixty Wistar rats were divided into four groups, namely the control group (without MTB induction) and the MTB-induced group (observations at week-3, week-6, and week-12 post infection). Induce tuberculosis with bacterial strain H37Rv ATCC 27294. Results: The number and size of the granuloma increased to a peak at week 6 and was consistent for weeks 6 and 12 post-infection. The kinetics of granulomas were consistent with IFN-γ and IP-10 levels. Conclusion: It was concluded that the model of tuberculosis infection by the H37Rv ATCC 27294 strain in Wistar rat found granuloma characteristics and IFN-γ and IP-10 patterns in similar kinetics, so that there was involvement of these molecules in TB pathology. Thus, tuberculosis infection of the H37Rv ATCC 27294 strain in rats can serve as a model for rapid tuberculosis study and display a complete pathological phase.
Keywords: granuloma, cytokines, chemokines, strain H37Rv ATCC 27294, rats model.

1. BACKGROUND

Every year an estimated 1.4 million cases of tuberculosis (TB) occur in the world (1). Improved access and treatment are critical to controlling the problem. Molecular diagnostic tests, although available, are not feasible in developing countries. Where available, these tests require state-of-the-art laboratories and are not cheap. One alternative to molecular diagnostics is the use of acute phase protein values. This protein is a protein whose levels will increase or decrease in plasma in response to injury or inflammation (2).

Acute phase serum levels are elevated in tuberculosis patients. Of these proteins, CRP is often used clinically as an adjuvant test for diagnosis, especially in children. Interferon -induced protein 10 (IP-10) is a chemokine expressed by antigen-presenting cells in response to IFN-γ. By binding to the CXCR3 receptor, T cell migration will occur to the site of inflammation. IP-10 increases in both pediatric and adult TB cases (3-6). IP-10 is detected in the urine of active TB patients and decreases with treatment (7). Thus, IP-10 has the potential for monitoring activity and response to treatment (8-10). Another study proved that IP-10 was higher in patients with active and latent TB than in healthy subjects. In addition, the levels are higher in latent TB than in active TB (11). IP-10 was also significantly associated with bacterial load in sputum (9, 12, 13). Despite the potential, until now study using IP-10 as a diagnostic tool has not conducted a disease spectrum study (2). This leads to the need for studies with animal models to assess the profile of IFN-γ and IP-10 in the pathological course of tuberculosis. Therefore, this in vivo study aimed to evaluate the pathology of granulomas and changes in IFN-γ and IP-10 levels in the progression of tuberculosis in animal models.

2. OBJECTIVE

This study aimed to analyze the kinetics of granulomas and the role of IFN-γ and IP-10 in the pathology of tuberculosis in a rat model.
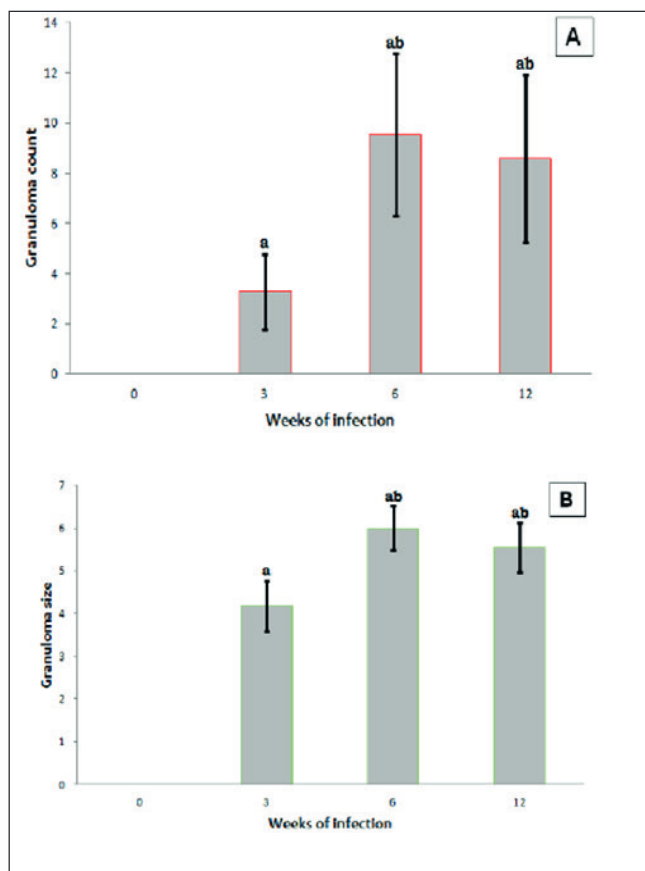
Figure 1. The number (A) and size (B) of granuloma from all group. a: p < 0.05 in comparison with the control group; a: p < 0.05 in comparison with the tuberculosis group at weeks-3 of observation.

3. MATERIAL AND METHODS

3.1 Animals

We were used male Wistar strains with body weights of 200–250 grams procured from the Bogor Veterinary Research Institute's animal house in West Java, Indonesia. Rats were housed in biosafety level 3 cages at the Veterinary Research Institute in Bogor, West Java, Indonesia. Throughout the experiment, rat were fed regular chow and given unlimited access to water.

3.2 Tuberculosis rat model

H37Rv ATCC 27294 was the bacterial strain utilized to create this tuberculosis model. An inhalation approach was used to cause pulmonary tuberculosis. Aerosolids were used to inhale bacteria for 30 minutes. The bacterium concentration was 105 CFU/lung. Sixty Wistar rats were divided into four groups, namely the control group (without MTB induction) and the MTB-induced group (observations at week-3, week-6, and week-12 post infection). Induce tuberculosis with bacterial strain H37Rv ATCC 27294.

3.3. Lung and blood sampling

The anesthetic ketamine was injected intramuscularly into the rat thigh muscle for surgery. Blood samples were collected via the posterior vena cava after surgery in the thoracic area. Lung organ was then isolat-

ed and histologically processed. When the heart was still beating, blood was extracted. The blood is then placed in a vacutainer to isolate the serum. For 15 minutes, blood samples were centrifuged at 3000 rpm. The serum was stored at -40oC overnight before being moved to -80oC the next day for evaluation. All lobes of the lung were sampled.

3.4. Cytokines analysis

An enzyme-linked immusorbent assay approach was used to analyze IFN-γ and IP-10 in serum. We use Rat Interferon Gamma ELISA kit (catalog No. BZ-08183010-EB) and Interferon-Inducible Protein 10 ELISA kit (catalog No. BZ-08183420-EB) which purchased from Bioenzy (Jakarta, Indonesia). The analysis technique was carried out in accordance with the kit's protocol.

3. 5. Ethics

All experimental animals were kept in facilities according to protocols approved by the research ethics committee of the Faculty of Medicine, Sebelas Maret University, Surakarta, Central Java, Indonesia.

4. RESULTS

Figure 1 depicts the frequency and size of granulomas from distinct groups. When compared to the control, the number and size of granulomas increased considerably at weeks 3, 6, and 12 (p < 0.05). The number and size of marophages increased at week-6 compared to week-3 (p < 0.05). The number and size of granulomas at week 6 did not differ substantially from those at week 12 (p > 0.05).

Table 1 shows the IFN-γ levels in various groups. When compared to controls, IFN-γ, levels increased at weeks 3, 6, and 12 post infection compared the control (p < 0.05). IFN-γ levels were substantially greater at week-6 than at week-3 or week-12 (p < 0.05). There was a substantial decrease in IFN-γ, levels after 12 weeks compared to 3 or 6 weeks (p < 0.05).

Table 2 shows the IP-10 chemokine levels from the various observation groups. There was an increase in IP-10 levels in all infection groups compared to controls (p < 0.05). IP-10 levels increased significantly at week-6 compared to week-3 (p < 0.05). There was a significant decrease in IP-10 at week-12 compared to week-6 or week-3 (p < 0.05).

Cytokine	Control	Weeks 3	Control	Weeks 6	Control	Weeks 12
IFN-γ	105.45 ± 3.57	130.61 ± 3.85[a]	105.92 ± 5.08	142.55 ± 4.17 [ab]	95.09 ± 4.64	118.38 ± 5.36[abc]

Table 1.The levels of cytokine in each weeks of observationNote: 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.

Cytokine	Control	Weeks 3	Control	Weeks 6	Control	Weeks 12
IP-10	481.79 ± 10.12	680.45 ± 21.79[a]	484.36 ± 11.35	726.20 ± 7.64[ab]	482.5 ± 7.41	530.39 ± 20.80[abc]

Table 2. The levels of IP-10 chemokine in each weeks of observation. Note: 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.

5. DISCUSSION

Experimental animals are very important to understand the pathogenesis and immunity of tuberculosis. This is based on the characteristics of M. tuberculosis infection which is very heterogeneous at the level of pathogenesis and immunity. Non-human primates are very good at recapitulating the immune response and sensitivity to TB. As for the smaller animal models, mice, rats, guinea pigs, rabbits, and zebra fish are more useful for investigating aspects of granuloma formation, sensitivity to different strains, or pre-clinical vaccine response.

In this study, we used mice as animal models to examine lung pathology and latency. Various mouse strains have been used to model tuberculosis (14-16). Previous findings have proven that model mice display physiological and pharmacological characteristics of the model (17). The lung pathology that we got from the Wistar mouse model was the formation of granulomas, which increased significantly at 3 weeks, 6 weeks, and 12 weeks post-infection compared to controls. The formation of granulomas will be stable from 6 to 12 weeks post-infection. We suspected that the bacterial strain we used (H37Rv ATCC 27294) reached a latent phase starting at the sixth week. Previous research on the Beijing W4 bacterial strain found that M. tuberculosis infection would become germ-free within 4 months (18, 19). Our study is consistent with previous findings using the same strain that the bacterial load was constant at 12 weeks post-infection (20).

IFN-γ is very important for mononuclear cell inflammation (21). Inflammatory cells activated by cytokines lead to the formation of granulomas containing pathogens (22, 23). In this study it was revealed that IFN-γ peaked at week 6 and then decreased at week 12 post-infection. This pattern is based on the capacity of IFN-γ in granuloma formation, which reaches its maximum at week 6 and then stabilizes until week 12 post-infection.

In this study we found the same pattern between IFN-γ and IP-10. This indicates that the expression of IFN-γ and IP-10 has the same profile. IP-10 is a chemokine expressed by antigen presenting cells in response to IFN-γ. Binding to the CXCR3 receptor will trigger T cell migration to the site of infection (2, 4. 8, 24). The level of IP-10 is higher than IFN-γ so it has the opportunity as a marker of disease detection and monitoring (8, 25). Our study agrees with the theory that IP-10 levels are higher than IFN-γ and have similar secretory dynamics. It is interesting that there was a decrease in the secretion of both markers at week 12. This shows that the strain that we used has a faster latency time than the other strains. The Beijing W4 strain gained a latency of 4 months (18). In addition, it was found that there was an increase in IP-10 in the three weeks of observation compared to controls. This indicates that IP-10 is involved in the development of pulmonary tuberculosis in a biphasic pattern, increasing at maximal levels in 6 weeks and then decreasing at 12 weeks. IP-10 is a potent chemoattractant for attracting monocytes and T cells to sites of inflammation. The increase at week-3 and peak at week-6 is intended to in-hibit the growth of germs. This strengthens the results of previous studies, which proved the function of IP-10 as an inhibitor of the growth of MTB bacteria in the ex vivo system (26).

6. CONCLUSION

It was concluded that the model of tuberculosis infection by the H37Rv ATCC 27294 strain in mice found granuloma characteristics and IFN-γ and IP-10 patterns with similar kinetics, so there was involvement of these molecules in TB pathology. Thus, tuberculosis infection of the H37Rv ATCC 27294 strain in mice can serve as a model for rapid tuberculosis research and display a complete pathological phase.

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