



UNIVERSITI PUTRA MALAYSIA

**EFFECTS OF INTERLEUKIN-18 MODULATION ON THE PATHOGENESIS
OF MALARIA INFECTION**

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By

MARZIEH JABBARZARE



**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
the Fulfillment of the Requirements for Degree of Master of Science**

March 2013

DEDICATION

In the name of God, the most beneficent and the most Merciful.

*This thesis is dedicated to
my dearest parents, Mohammad Ali and Fatemeh
for their immense support, patience, and encouragement
during all these years of the study.*

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of
the requirement for the degree of Master of Science

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Faculty : Medicine and Health Sciences

Malaria is an important parasitic infectious disease that afflicts mankind globally. The involvements of cytokines in the pathogenesis of malaria have well been documented and it has also been widely accepted that pro-inflammatory cytokines release plays crucial roles in the progression of severe pathology in malaria. Interleukin 18 (IL-18) is an important mediator which functions as an immune regulator and inducer of pro-inflammatory cytokines release. Activation of IL-18 in the immune system has been shown to amplify inflammatory responses in many disease conditions and may play a crucial role in the development of certain disease. This study is designed to determine the role and involvement of IL-18 during malaria infection and the effects of modulating its release on the course of the infection, the release of major pro- and anti-inflammatory cytokines and also the histopathological consequences in major affected organs during the infection. Male ICR mice infected with *Plasmodium berghei* (*P. berghei*) ANKA were used as malaria model in this study. Mice were injected intraperitoneally with 2 x

10^7 infected red blood cells. Plasma IL-18 concentrations in malaria-infected mice as measured by ELISA method, showed continuous increment from the beginning to the end of the infection. Release phase was found to be dependent on the level of severity of the infection. Modulation of the release of IL-18 was carried out by treating the malaria infected mice with recombinant mouse IL-18 (rmIL-18) and recombinant mouse IL-18 Fc chimera (rmIL-18 Fc chimera) intravenously. Inhibition of IL-18 release by rmIL-18 Fc chimera have delayed the emergence of the physical signs of infection and the development of parasitemia, subsequently prolonging the life span of mice infected with malaria. Augmentation of systemic IL-18 with rmIL-18 significantly decreased the release of anti-inflammatory cytokine (IL-10), whereas, inhibition of IL-18 with rmIL-18 Fc chimera showed increased level of IL-10. A significant elevation of pro-inflammatory cytokines (TNF α , IFN γ , IL-1 α and IL-6) was also observed during augmentation of IL-18 level. Nonetheless, these pro-inflammatory cytokines decreased during inhibition of IL-18 by rmIL-18 Fc chimera. From the pattern of cytokines release, it can be suggested that IL-18 exerts a pro-inflammatory activity in the Th1 type response by signaling the production of IFN γ during malaria. Histopathological analysis performed on major organs known to be affected during malaria infection which include the brain, lungs, liver, spleen and kidneys of malarial mice showed significant histopathological changes in all organs of malarial mice. Treatment with rmIL-18 Fc chimera showed significant improvement on the histopathological conditions of the organs as compared to the malarial mice treated with PBS. However, mice treated with recombinant mouse IL-18 showed severe and worsening histopathology during the infection. In conclusion, the results from this study suggest that IL-18 may well play a

crucial role in mediating the severity of malaria infection and it may play a key pro-inflammatory role throughout immune response against the disease. Antagonizing IL-18 activity has improved the histopathological conditions associated with the disease which may well suggest that targetting IL-18 would provide a potentially significant therapeutic benefit in malaria therapy.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai
memenuhi keperluan untuk ijazah Master Sains

KESAN MODULASI INTERLEUKIN-18 TERHADAP PATOGENESIS JANGKITAN MALARIA

Oleh

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Malaria merupakan penyakit jangkitan parasit yang memberikan masalah kepada manusia secara global. Penglibatan sitokin dalam pathogenesis malaria telah didokumentasikan dengan jelas dan ianya telah diterima secara meluas bahawa pembebasan sitokin proinflamasi memainkan peranan penting dalam perkembangan patologi tenat malaria. Interleukin-18 (IL-18) merupakan suatu perantara penting yang berfungsi sebagai pengawalatur imun dan perangsang pembebasan sitokin pro-inflamasi. Pengaktifan IL-18 dalam sistem imun telah dilapor menguatkan gerakbalas inflamasi dalam banyak penyakit dan mungkin memainkan peranan penting dalam perkembangan penyakit-penyakit tertentu. Kajian ini direkabentuk bagi menentukan peranan dan penglibatan IL-18 semasa jangkitan malaria dan kesan modulasi pembebasannya ke atas keadaan jangkitan, pembebasan sitokin pro- dan anti-inflamasi utama dan juga kesan histopatologi dalam organ-organ utama yang terlibat semasa jangkitan. Mencit ICR jantan yang dijangkiti *Plasmodium berghei* (*P. berghei*) ANKA digunakan sebagai

model malaria dalam kajian ini. Mencit disuntik secara intraperitoneal dengan 2×10^7 sel darah merah terjangkit. Kepakatan plasma IL-18 dalam mencit yang dijangkiti malaria seperti yang disukat menggunakan cara ELISA, menunjukkan peningkatan berterusan dari permulaan sehingga ke akhir jangkitan. Fasa pembebasan didapati bergantung kepada tahap ketenatan jangkitan. Modulasi pembebasan IL-18 dilakukan dengan merawat mencit yang dijangkiti malaria dengan IL-18 mencit rekombinan (rmIL-18) dan IL-18 Fc kimera mencit rekombinan (rmIL-18 Fc) secara intravena. Perencutan pembebasan IL-18 oleh rmIL-18Fc telah melewatkkan kemunculan tanda-tanda fizikal jangkitan dan perkembangan parasitaemia, seterusnya memanjangkan tempoh hayat mencit yang dijangkiti malaria. Penambahan IL-18 sistemik dengan rmIL-18 menurunkan pembebasan sitokin anti-inflamasi (IL-10) secara signifikan, manakala perencutan IL-18 dengan rmIL-18 Fc menunjukkan peningkatan tahap IL-10. Peningkatan signifikan sitokin pro-inflamasi (TNF α , IFN γ , IL-1 α dan IL-6) juga diperhatikan semasa penambahan tahap IL-18. Walau bagaimana pun, sitokin pro-inflamasi ini menurun semasa perencutan IL-18 dengan rmIL-18 Fc kimera. Dari corak pembebasan sitokin, boleh dicadangkan bahawa IL-18 menghasilkan aktiviti pro-inflamasi dalam gerakbalas jenis Th1 dengan mengisyaratkan pengeluaran IFN γ semasa jangkitan. Analisis histopatologi yang dijalankan ke atas organ-organ utama yang diketahui terkesan semasa jangkitan malaria termasuk otak, paru-paru, hati, limpa dan ginjal menunjukkan perubahan histopatologi yang signifikan dalam kesemua organ mencit yang dijangkiti malaria. Rawatan dengan rmIL-18 Fc kimera menunjukkan pemberian signifikan ke atas keadaan histopatologi organ-organ berbanding dengan mencit terjangkit malaria yang dirawat dengan PBS. Walau bagaimana pun, mencit yang dirawat dengan IL-18 mencit rekombinan menunjukkan histopatologi yang lebih teruk

dan tenat semasa jangkitan. Kesimpulannya, keputusan dari kajian ini mencadangkan bahawa IL-18 mungkin memainkan peranan yang penting dalam mengantarakan ketenatan jangkitan malaria dan ia mungkin memainkan peranan pro-inflamasi utama sepanjang gerakbalas imun terhadap penyakit ini. Penentangan aktiviti IL-18 telah memperbaiki keadaan histopatologi yang dikaitkan dengan malaria di mana ini mungkin mencadangkan bahawa mensasarkan IL-18 boleh menghasilkan keuntungan terapeutik ketara yang berpotensi dalam terapi malaria.

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I certify that a Thesis Examination Committee has met on **Date** to conduct the final examination of Marzieh Jabbarzare on her Master of Science thesis entitled "**Effects of interleukin-18 modulation on the pathogenesis of malaria infection**" in accordance with Universiti Pertanian Malaysia Act 1980 and Universiti Pertanian Malaysia regulations 1981. The Committee recommends that the student be awarded the Master of Science

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

MARZIEH JABBARZARE

Date:



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