UNIVERSITI PUTRA MALAYSIA

ROLE, INVOLVEMENT AND POTENTIAL OF INTERLEUKIN-27 AS AN IMMUNOTHERAPEUTIC TARGET IN MURINE MALARIA

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By

SITI SARAH BINTI FAZALUL RAHIMAN

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

August 2012

In the name of Allah, the most Compassionate and the Most Merciful.

To my beloved husband, thank you for your endless support.

To my supportive parents and parents-in-laws, brothers and sisters,
thank you for your unconditional love.

to Prof Madya Dr Abas Hj Hussin, may Allah ease your burdens
and each day is a little bit brighter.

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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SITI SARAH BINTI FAZALUL RAHIMAN

August 2012

Chair : Rusliza binti Basir, PhD

Faculty : Faculty of Medicine and Health Sciences

Interleukin-27 (IL-27) has been known to exert pleiotropic role in many inflammatory-related diseases including parasitic infection. However, its involvement during malaria infection has yet to be elucidated. In this study, the role and involvement of IL-27 during malaria infection was investigated and the effects of modulating its release on the course of the infection, the release of major inflammatory cytokines and the histopathological consequences in major affected organs during malaria were evaluated. *Plasmodium berghei (P. berghei)* ANKA infection in male ICR mice was used as a model for malaria infection. The mice were inoculated intraperitoneally with 2 x 10^7 parasite-infected red blood cells (PRBCs) whereas the controls received an equivalent dilution of normal RBCs. The concentration of IL-27 in the plasma of malarial mice measured by means of ELISA, showed persistent elevation of IL-27 right from the early until the late phase of infection and its release is independent of the degree of disease severity. The modulation of IL-27 release was carried out by treatment of malarial mice with recombinant mouse IL-27 (rmIL-27), WSX-1Fc chimera or anti-WSX-1 monoclonal

antibody (WSX-1 mAb) intravenously. Inhibition of IL-27 with WSX-1 Fc chimera and WSX-1 antibodies delayed the appearance of physical signs of illness and parasitaemia development and also prolonged the survival of malaria-infected mice. Augmentation of IL-27 with rmIL-27 significantly elevated the release of anti-inflammatory cytokine (IL-10) whereas inhibition and neutralisation of IL-27 with WSX-1 Fc chimera and WSX-1 mAb respectively, showed decreased level of IL-10. A significant elevation of pro-inflammatory cytokines (IFN-γ and IL-6) was also observed, both during augmentation and inhibition of IL-27. From the pattern of cytokines release, it can be suggested that IL-27 exerts an anti-inflammatory activity in the T_h1 type response by signalling the production of IL-10 during malaria. Histopathological examination performed on internal organs including the brain, lungs, liver, spleen and kidneys of malarial mice showed significant sequestration of PRBCs in the microvasculature of all the major organs of malarial mice. Other significant histopathological changes were also observed in malarial mice such as hyperplasia and hypertrophy of the Kupffer cells in the liver, hyaline membrane formation in the lungs, enlargement of the white and red pulp element followed by the lost of structure of germinal centre in the spleen, and vacuolation of the kidney tubule cells. Treatment with rmIL-27 and WSX-1 Fc chimera failed to show any significant improvement on the histopathological conditions of the organs of malaria-infected mice. Overall, the results suggest that IL-27 is involved during malaria infection and it may play an important anti-inflammatory role during immune response against the disease. Modulation of its release produced a positive impact on inflammatory cytokine production during the infection, suggesting its potential as an immunotherapeutic target in malaria, in which the host may benefit from its inhibition.

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

PERANAN, PENGLIBATAN DAN POTENSI INTERLEUKIN-27 SEBAGAI SASARAN IMMUNOTERAPEUTIK DALAM MALARIA

Oleh

SITI SARAH BINTI FAZALUL RAHIMAN

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Fakulti : Fakulti Perubatan dan Sains Kesihatan

Interleukin-27 (IL-27) telah diketahui mempunyai peranan pleiotropik dalam banyak penyakit berkaitan inflamasi termasuk jangkitan parasit. Walau bagaimanapun, penglibatannya semasa jangkitan malaria masih belum diperjelaskan. Dalam kajian ini, peranan dan penglibatan IL-27 dalam jangkitan malaria telah diselidiki dan kesan modulasi penghasilannya ke atas keadaan keseluruhan jangkitan malaria, pembebasan sitokin inflamasi utama dan perubahan histopatologi organ-organ utama yang terjejas semasa jangkitan malaria telah dinilai. Jangkitan *Plasmodium berghei* (*P. berghei*) ANKA dalam mencit ICR jantan telah digunakan sebagai model jangkitan malaria. Mencit disuntik secara intraperitoneal dengan 2 x 10^7 sel darah merah berparasit. Kepekatan IL-27 dalam plasma mencit yang dijangkiti malaria yang diukur melalui kaedah ELISA, menunjukkan peningkatan berterusan IL-27 bermula dari awal hingga ke akhir fasa jangkitan dan pembebasannya didapati tidak bergantung kepada tahap ketenatan jangkitan. Modulasi ke atas pembebasan IL-27 dijalankan dengan merawat mencit yang dijangkiti malaria dengan rekombinan mencit IL-27 (rmIL-27), WSX-1Fc chimera dan antibodi monoklonal anti-WSX-1

secara intravena ke atas mencit yang dijangkiti malaria. Perencatan pembebasan IL-27 oleh WSX-1 Fc chimera dan antibodi WSX-1 memperlahankan kemunculan tanda-tanda fizikal jangkitan dan perkembangan parasitemia seterusnya melanjutkan tempoh hayat mencit yang dijangkiti malaria. Penambahan IL-27 dengan rmIL-27 meningkatkan pembebasan sitokin anti-inflamasi (IL-10) secara signifikan manakala perencatan dan peneutralan IL-27 masing-masing dengan WSX-1 Fc chimera dan antibodi WSX-1, menunjukkan penurunan tahap IL-10. Peningkatan signifikan sitokin proinflammasi (IFN-γ dan IL-6) juga diperhatikan semasa penambahan dan perencatan IL-27. Daripada corak pembebasan sitokin-sitokin, boleh diusulkan bahawa IL-27 menunjukkan aktiviti anti-inflamasi dalam gerakbalas jenis T_h1 dengan mengisyaratkan penghasilan IL-10 semasa malaria. Pemeriksaan histopatologi yang dilakukan ke atas organ-organ dalaman termasuk otak, paru-paru, hati, limpa dan ginjal menunjukkan sekuestrasi sel-sel darah merah berparasit dalam mikrovaskulatur kesemua organ-organ utama mencit yang dijangkiti malaria. Perubahan histopatologi lain yang signifikan juga diperhatikan dalam mencit yang dijangkiti malaria termasuk hiperplasia dan hipertrofi sel-sel Kupffer dalam hati, pembentukan membran hyalin dalam paru-paru, pembesaran elemen pulpa merah dan putih diikuti dengan kehilangan struktur pusat germinal dalam limpa, dan vakuolasi sel-sel tubul ginjal. Rawatan dengan rmIL-27 dan WSX-1 Fc chimera gagal menunjukkan sebarang kesan pembaikan yang signifikan ke atas keadaan histopatologi organ-organ mencit yang dijangkiti malaria. Secara keseluruhannya, keputusan kajian mencadangkan penglibatan IL-27 semasa jangkitan malaria dan ianya mungkin memainkan peranan antiinflamasi yang penting semasa gerakbalas imun melawan jangkitan malaria. Modulasi pembebasannya menghasilkan impak yang positif ke atas pembebasan sitokin inflamasi semasa jangkitan, dan ini

mencadangkan potensinya sebagai sasaran imunoterapeutik di mana hos boleh memperolehi manfaat dari perencatannya.

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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.

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