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EFFECTS OF AGE ON MICROGLIAL RESPONSES TO CELLULAR STRESS INDUCED BY LIPOPOLYSACHARIDES AND BETA AMYLOID

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By

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Microglia are the resident macrophages of the central nervous system (CNS). In the normal CNS, they are in a resting condition, characterised by low expression of MHC class II (MHC II) and co stimulatory molecules such as CD40. Following activation by various stressors, microglia acquire an inflammatory phenotype and the continuous activation of microglia is thought to exacerbate neuronal damage. It is also believed that with increasing age, the inflammatory response of microglia becomes uncontrolled. In this study, the effects of age on microglia responses were determined by culturing microglia from Sprague Dawley rats of 6 days (neonates), 2-4 months (adult) and 3 years (old). Microglia were then activated with lipopolysaccharide (LPS; 1μg/ml) or beta amyloid (Aβ; 25 or 50μg/ml) and assessed for expression of CD40 and MHC II activation markers, nitric oxide (NO) production and proliferative capacity at pre-determined time

points. Adult microglia were difficult to culture, therefore optimisations were carried out to support and enhance cell growth by coating culture flasks with poly-L-lysine and supplementing culture with insulin-transferrin-selenium (ITS) and macrophage colony stimulating factor (M-CSF). Expression of CD40 and MHC II was determined by immunophenotyping. Basal expression of CD40 and MHC II was higher in older microglia compared to neonates. Similarly, after activation with LPS and Aβ, CD40 and MHC II expression was higher in older cells. Upon LPS stimulation, the CD40 and MHC II expression was as follows: neonates, 11.3 ± 1.8% and 4.2 ± 2.3%; 2-4 months, 32.4 ± 25.2% and 21.2 ± 8.8%; 3 years, 26.4 ± 15.3% and 21.1 ± 12.6% respectively. Upon stimulation with Aβ, the CD40 and MHC II expression was: neonates, 50.3 ± 19.8% and 3.6 ± 1.4%; 2-4 months, 63.9 ± 24.5% and 36.9 ± 7.4%; 3 years, 75.2 ± 24.1% and 15.1 ± 11.5% respectively. The higher expression of CD40 and MHC II in older microglia is indicative of its potential for subsequent activation of T cells. Using the Griess assay and [^{3}H]-thymidine proliferation assay, it was observed that the NO production and proliferation rate was induced at 48hrs and 18hrs time point respectively. Under resting condition, older microglia released marginally higher NO compared to neonates ($p<.05$); however, upon stimulation, older microglia released significantly lower ($p<.05$) NO production compared to neonates. Under resting condition, the NO production was as follows: neonates, 2.0μM; 2-4 months, 6.9μM and 3 years, 5.3μM. Upon stimulation with LPS and 50μg/ml Aβ, the NO production was: neonates, 29.3 and 60.2μM; 2-4 months, 11.9 and 9.9μM; 3 years, 9.9 and 5.7μM respectively. As for the proliferation capacity,

older cells displayed significantly lower proliferation ($p<.05$) both when resting and upon stimulation, compared to neonates. In the resting condition, 2-4 months and 3 years old microglia recorded readings of 39929 cpm and 37328 cpm lower compared to neonates. Upon stimulation with LPS and 50μg/ml Aβ, proliferation rate of older microglia were consistently lower compared to neonates. Thus, as a result of our work, we demonstrated differences between primary neonatal and adult microglia responses to LPS and Aβ stimulation by comparing activation profiles, NO and proliferation rate. We demonstrated that although older cells exhibit a higher activation state upon stimulation (based on CD40 and MHC II expression), their functional aspects such as NO production and proliferation remain significantly low compared to neonates.

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

KESAN UMUR KE ATAS GERAK BALAS SEL MIKROGLIA TERHADAP TEGASAN SEL YANG DIRANGSANG OLEH LIPOPOLISAKARIDA DAN BETA AMILOID

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Mikroglia ialah makrofaj yang terdapat di sistem saraf pusat. Mikroglia yang berada dalam keadaan rehat dalam sistem saraf pusat normal didirikan oleh tahap penghasilan kompleks histokompatibiliti utama kelas II (MHC II) dan molekul ko-perangsang CD40, yang rendah. Mikroglia akan menunjukkan fenotip keradangan selepas diaktifkan oleh stresor. Pengaktifan mikroglia secara berterusan akan menyebabkan kerosakan neuron. Faktor usia dipercayai akan menyebabkan mikroglia menghasilkan gerak balas keradangan yang tidak terkawal. Dalam kajian ini, kesan faktor usia ke atas gerak balas mikroglia dilakukan dengan kultur sel mikroglia tikus Sprague Dawley berusia 6 hari, 2-4 bulan dan 3 tahun. Mikroglia kemudian diaktifkan dengan lipopolisakarida (LPS) atau beta amiloid (Aβ) dan ekspresi penanda

pengaktifan, penghasilan nitrik oksida (NO) dan kapasiti kembangbiak dinilai pada sela masa yang ditentukan. Ekspresi penanda pengaktifan, MHC II dan CD40, ditentukan oleh teknik imunofenotip. Mikroglia dewasa sukar untuk dikultur, oleh itu, pengoptimuman telah dilakukan untuk menggalakkan pertumbuhan sel dewasa secara melapik kelalang kultur menggunakan faktor pelekatan (poly-L-lysine) dan menambah faktor pertumbuhan; insulin-transferrin-selenium (ITS) serta faktor perangsang koloni (M-CSF). Didapati ekspresi basal CD40 dan MHC II adalah tinggi pada mikroglia dewasa berbanding neonatal. Selanjutnya, selepas diaktifkan LPS dan Aβ, ekspresi CD40 dan MHC II adalah lebih tinggi pada mikroglia dewasa. Setelah diaktifkan dengan LPS, ekspresi CD40 dan MHC II adalah seperti berikut: neonatal, 11.3 ± 1.8% dan 4.2 ± 2.3%; 2-4 bulan, 32.4 ± 25.2% dan 21.2 ± 8.8%; 3 tahun, 26.4 ± 15.3% dan 21.1 ± 12.6% masing-masing. Setelah diaktifkan dengan Aβ, ekspresi CD40 dan MHC II adalah seperti berikut: neonatal, 50.3 ± 19.8% dan 3.6 ± 1.4%; 2-4 bulan, 63.9 ± 24.5% dan 36.9 ± 7.4%; 3 tahun, 75.2 ± 24.1% dan 15.1 ± 11.5% masing-masing. Ini menunjukkan mikroglia dewasa mungkin mengekspreskan isyarat pempamer antigen dan ko-perangsang, yang juga menunjukkan potensi untuk mengaktifkan sel T. Penghasilan NO dan kadar kembangbiak sel diperhatikan dengan menggunakan cerakin Griess dan [^{3}H]-timidina selepas diaruhkan selama 48 jam dan 18 jam, masing-masing. Dalam keadaan rehat, mikroglia yang dewasa merembeskan NO lebih tinggi secara signifikan berbanding neonatal ($p<.05$); Walau bagimanapun, selepas diaruh, mikroglia dewasa merembeskan NO lebih rendah secara signifikan ($p<.05$) berbanding neonatal. Dalam keadaan rehat, rembesan NO adalah seperti berikut: neonatal, 2.0 μM; 2-

4 bulan, 6.9 μM dan 3 tahun, 5.3 μM. Setelah diaktifkan dengan LPS dan 50μg/ml Aβ, rembesan NO adalah seperti berikut: neonatal, 29.3 dan 60.2μM; 2-4 bulan, 11.9 dan 9.9μM; 3 tahun, 9.9 dan 5.7μM masing-masing. Bagi kapasiti kembangbiak, sel dewasa berkembangbiak lebih rendah secara signifikan ($p<.05$) ketika dalam keadaan rehat dan selepas diaruhkan, berbanding neonatal. Dalam keadaan rehat, kapasiti kembangbiak neonatal adalah 55617 kiraan per minit (cpm) tetapi mikroglia dewasa iaitu 2-4 bulan dan 3 tahun menunjukkan 39929 cpm dan 37328 cpm lebih rendah berbanding neonatal. Setelah diaktifkan oleh LPS dan 50μg/ml Aβ, kapasiti kembangbiak neonatal adalah 48847 cpm dan 19007 cpm masing-masing tetapi mikroglia dewasa iaitu 2-4 bulan menunjukkan 12497 cpm (LPS) dan 10334 cpm (Aβ) lebih rendah berbanding neonatal. Mikroglia dewasa, iaitu 3 tahun juga menunjukkan 30609 cpm (LPS) and 8668 cpm (Aβ) lebih rendah berbanding neonatal. Oleh itu, berdasarkan hasil, kami menunjukkan perbezaan gerak balas antara neonatal primer dan mikroglia dewasa terhadap pengaruhan LPS dan Aβ dengan membandingkan profil pengaktifan, NO dan kadar kembangbiak. Kami juga menunjukkan, meskipun sel dewasa mempamerkan tahap pengaktifan yang tinggi selepas diaruh (berdasarkan ekspresi CD40 dan MHC II), aspek fungsi seperti penghasilan NO dan kadar kembangbiak masih rendah secara signifikan ($p<.05$) berbanding neonatal.

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I certify that an Examination Committee has met on (24th March 2011) to conduct the final examination of Hemavathy Subramaiam on her Master thesis entitled "Effects of age on microglia responses to cellular stress induced by lipopolysacharide (LPS) and beta amyloid (Aβ)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master degree.

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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.

HEMAVATHY SUBRAMAIAM

Date: 24 March 2011

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