UNIVERSITI PUTRA MALAYSIA

IMMUNOMODULATORY EFFECTS OF HUMAN MESENCHYMAL STEM CELLS ON NEUTROPHIL FUNCTIONS

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By

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

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Abstract of the Thesis Presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

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Chairman : Rajesh Ramasamy, PhD

Faculty : Medicine and Health Sciences

Polymorphonuclear neutrophil (PMN) are common professional phagocytic cells of the innate immune system. In modern medicine the functions of neutrophils go far beyond the classical phagocytosis and pathogen killing. They perform sophisticated regulatory functions, having implications not only in the inflammatory and immune responses but also in haematopoiesis, wound healing and antimicrobial activities. Neutrophils mediate immune responses that contribute to tissue repair and damage. However, their over activation can lead to detrimental effects. To maintain normal tissue homeostasis, the dual roles of neutrophils are important but they need to be carefully modulated.

Mesenchymal stem cells (MSC) are non-haematopoietic, multipotent cells that exert immunomodulatory activity on immune cells. MSC have been shown to interact with innate and adaptive immune cells by modulating their functional responses *in vitro* and *in vivo.* The goal of our study was to further build upon these findings by determining the MSC specific immunomodulatory effects on the neutrophil functions. We went about this task by isolating Human neutrophils from whole blood using an optimised Ficoll-dextran method and freshly isolated neutrophils were used in all experiments. Neutrophils in the presence or absence of MSC were assessed for viability, cellular proliferation, chemotaxis, phagocytosis, respiratory burst and apoptosis activities. The multistep optimised isolation method yielded recovery of >50% neutrophils which was confirmed by Leishman staining with purity of >95%. Mesenchymal stem cells significantly (* $P<0.05$) enhanced the viability of resting and phorbol myristate acetate (PMA) activated neutrophils and simultaneously rescued both resting and PMA activated neutrophil from apoptosis. In terms of neutrophil effector functions MSC significantly (* $P<0.05$) inhibited opsonised zymosan (OZ) and lipopolysaccharide (LPS) induced neutrophil phagocytosis and also significantly (* $P<0.05$) inhibited resting, PMA, OZ, fMLP (f-Met-Leu-Phe, N-formylated peptides) and *E.coli* activated neutrophils respiratory burst. However MSC did not affect neutrophil chemotaxis in either resting or activated state. Similarly *in vitro* analysis also revealed MSC failed to induce any changes in basal cell proliferation activity of neutrophil. Overall the results reveal that MSC have an immunosuppressive effect on neutrophil survival, apoptosis,

phagocytosis and respiratory burst activity. These findings display the efficiency of MSC in limiting the hostile effects of neutrophils. Consequently this study makes a compelling case for the use of MSC as a therapeutic tool for the treatment of neutrophil mediated immune disorders.

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

KESAN KESAN SEL SEL INDUK MESENCHYMAL KE ATAS FUNGSI FUNGSI NEUTROFIL

Oleh

MARYAM MAQBOOL

Julai 2011

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Dalam perubatan moden, fungsi neutrofil menjangkaui fagositosis dan pembunuhan mikroorgansima penyebab penyakit. Neutrofil melakukan fungsi yang sofistikated mempunyai implikasi bukan sahaja dalam keradangan dan tindakbalas imun tetapi juga hematopoiesis, penyembuhan luka dan aktiviti antibakteria. Walaupun neutrofil mengubah sistem imun dan menyumbang kepada pembaikan tisu, pengaktifan yang berlebihan boleh membawa kepada kesan yang membahayakan. Dwi peranan neutrofil adalah penting dalam normal tisu homeostasis tetapi perlu dipantau. Sel-sel induk mesenkima (MSC) adalah bukan hematopoietik, sel multipoten yang mengerahkan aktiviti imunomodulator pada sel imun. MSC menunjukkan untuk berinteraksi dengan sel imun bawaan dan adaptif dengan modulasi fungsi mereka di in vitro dan in

vivo. Maka, kami Ingin menjelaskan fungsi imunotindakan oleh MSC pada neutrofil. Pengasingan neutrofil dibuat menggunakan kaedah ficol-dextran yang telah dioptimumkan dan neutrofil yang baru diasingkan dari darah digunakan dalam semua eksperimen. Neutrofil dengan kehadiran atau ketiadaan MSC telah dikaji untuk aktiviti-aktiviti: “viability”, “cellular proliferation”, “chemotaxis”, fagositosis, “respiratory burst” dan “apoptosis”. Kaedah pengasingan yang telah dioptimumkan telah memberikan lebih dari 95% neutrofil yang masih hidup yang disahkan dengan menggunakan teknik pewarnaan Leishman dan memberi lebih pemulihan 50% ke atas. MSC telah meningkatkan jangka hidup neutrofil sama ada dalam kaedaan rehat atau neutrofil yang telah di aktifkan dengan PMA daripada netrofil menjalani process apoptosis yang boleh menyebabkan sel mati. Neutrofil yang telah diinduksi oleh zymosan (OZ) dan lipopolysaccharide (LPS) telah dihalang daripada melalui process fagositosis oleh MSC. Tetapi MSC tidak memberi kesan kepada pergerakan kimia neutrofil dalam keadaan rehat atau dengan stimulasi. Proliferasi neutrofil juga dianalisis secara in vitro dan MSC juga tidak menujukkan sebarang respon ke atas proliferasi neutrofil. Keputusan menunjukkan MSC mempunyai kesan imunomodulatori terhadap keupayaan penghidupan neutrofil, “apoptosis”, fagositosis dan “respiratory burst”. Keputusan ini menunjukkan MSC boleh dijadikan bahan terapeutik yang menarik, untuk mengurangkan kesan buruk neutrofil dalam hal-hal kesihatan yang diganggu oleh neutrofil.

TO

My

Beloved Mother Dr Ezra Jamal

For her unconditional love, understanding, patience, support and encouragement that elevated my spirit

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In the name of Allah, the most gracious, most merciful. Praise is to Allah the cherisher and sustainer of the worlds. Show us the straight way and O my lord! Advance me in knowledge.

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I certify that an Examination Committee has met on _____ to conduct the final examination of **Maryam Maqbool** on her Master of Science thesis entitled **'IMMUNOMODULATORY EFFECTS OF HUMAN MESENCHYMAL STEM CELLS ON NEUTROPHIL FUNCTIONS'** in accordance with Universisti Putra Malaysia (Higher Degree) Act 1980 and Universiti Putra Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the degree of Master of Science.

Members of the Examination Committee were as follows:

HASANAH MOHD. GHAZALI, PhD
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This thesis was submitted to the School of Graduate Studies, Universiti Putra Malaysia, has been accepted as fulfilment of the requirements for the Degree of **Master of Science.** The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which has 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.

_____

MARYAM MAQBOOL

Date:

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