行政院國家科學委員會專題研究計畫 成果報告

急性肺損傷時之骨髓幹細胞對於肺部組織修復及纖維化的研究

研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 95-2745-B-002-004-
執 行 期 間 ：95 年 08 月 01 日至 96 年 07 月 31 日
執 行 單 位 ：國立臺灣大學醫學院內科

計 畫 主 持 人：郭律成

計畫參與人員：碩士班研究生-兼任助理：魏志純

處 理 方 式 ：本計畫可公開查詢

中 華 民 國 96 年 10 月 31 日

行政院國家科學委員會補助專題研究計畫 ■成果報告

急性肺損傷時之骨髓幹細胞對於肺部組織修復及纖維化的研究

計畫類別：■ 個別型計畫　　□ 整合型計畫
計畫編號：NSC 95－2745－B－002－004－
執行期間：　2006 年　8　月　1　日至　2007 年　7　月　31　日

計畫主持人：郭律成
共同主持人：無
計畫參與人員：

成果報告類型(依經費核定清單規定繳交)：■精簡報告　□完整報告

本成果報告包括以下應繳交之附件：
□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：

中　華　民　國　九十六　年　十　月 三十一　日

Introduction

Acute lung injury (ALI) resulted in decreased pulmonary compliance and compromised gas exchange, which lead to hypoxemia and subsequent multiple organ dysfunction. Currently, limited therapy is available for the management of ALI, except for mechanical ventilation and supportive care. ALI results in subsequent pulmonary fibrosis. Once the fibrotic process dominates, the outcome is poor.

Stem cells have both the capacity to self-renew and to differentiate along a specified molecular pathway. There are evidences that adult stem cells are involved in the process of organ injury or diseases, both in human and murine models. Marking the donor bone marrow by transgenic green fluorescence protein (GFP) is a commonly used method for tracing the mobilization of bone marrow stem cells.

Aims

1. Set up a murine model of bone marrow transplantation for stem cell study for ALI
2. Examine the extensiveness of bone marrow cell involvement in the process of ALI
3. Identify the lineage differentiation of bone marrow stem cell in the process of ALI

Methods

All animal procedures were carried out under ethical guideline.

Bone marrow transplantation

C57BL/6J mice were obtained and housed in the vivarium of our animal center. The animals were kept on a 12-h-on, 12-h-off light cycle and had access to chow and water *ad libitum.* Young adult female recipient mice with age 6-12 weeks and weighted over 20g underwent whole-body r-irradiation, with 5 Gray in a divided dose 3 hours apart, to ablate their bone marrow. Bone marrow of young adult male mice (C57BL/6J) at the same age were harvested from femurs and tibias by aspiration and flushing. Five million unfractionated bone marrow cells were injected into the tail vain of recipients. After the bone marrow transplantation (BMT), the mice were kept in sterile conditions for one month and fed with autoclaved feed *ad*

libitum. To verify successful engraftment, blood from the retro-orbital vein were collected at day 7 after BMT.

Acute lung injury model

Mice at 30 days post-BMT were anesthetized by intraperitoneal injection of 3.5% pantobarbitol. Lipopolysaccharide (LPS), which was used to induce acute lung injury, from *Escherichia coli* serotype O55:B5 were instilled intratracheally in a dose of 10 μg/20 microliter of saline per mouse. In the control group, PBS were instilled. The mice were killed by deeply anesthetizing with an intraperitoneal injection of 3.5% pantobarbitol, 0 (group 1), 1 (group 2), 3 (group 3), 5 (group 4), 7 (group 5), 14 (group 6) days after LPS instillation, and perfused transcardially with saline twice and then 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4, over a period of 15 minutes at room temperature. The thorax were opened and the lungs removed en bloc, and the lung allowed to fix in the same fixative for 24 hours. The lung tissue were embedded in paraffin or frozen for further study. Control lung tissue was obtained from healthy female with no acute lung injury (group 7). There will be 2 mice in each group.

Histological and Immunohistochemistry

Following final anaesthesia, animals were perfused with 4% paraformaldehyde at Day 0, 1, 3, 5, 7 and 2 weeks after transplantation. Lung sections (10-mm thick) were prepared with a cryotome (Leica, Nussloch, Germany). For immunohistochemistry, sections were treated with 4% paraformaldehyde(Sigma). Primary antibodies, applied at 4 degrees C overnight, were monoclonal Rabbit antibodies to Cytokeratin antigen (1:500, Santa Cruz), and Rat antibodies to CD45 antigen (1:500, Chemicon). Primary antibodies were visualized using corresponding Rodamin-conjugated secondary antibodies (chemicon) for 1h at room temperature (RT). Nuclei were counterstained with DAPI mounting medium (Vector). For control experiments, slices were stained with secondary antibodies only. Z-stacks were obtained using the multitracking mode of a LSM 5 Pascal laserscanning microscope (Zeiss, Jena, Germany) to avoid cross-talk between the red and green channels. Optical sections of 1 mm were obtained and analyzed by channel

emission profiles.

Results

At day 1, 4 and 8 after BMT, blood cells with GFP could be visualized at peripheral blood smear (Figure 1). It suggested the successful engraftment of transplanted GFP-positve cells. After intratracheal instillation of LPS to induce acute lung injury, positive GFP cells were observed in the lung parehchyma (Figure 2). Cytokeratins play a critical role in differentiation and tissue specialization and function to maintain the overall structural integrity of epithelial cells. It increased rapidly (at day 0) after induction of ALI, suggesting the start of repairing and differentiation process. CD45 was expressed on all hematopoietic cells. Its expression persisted through the period of observation with maximal value at day 5. The change of its amount may be due to differentiation of cells.

Figures

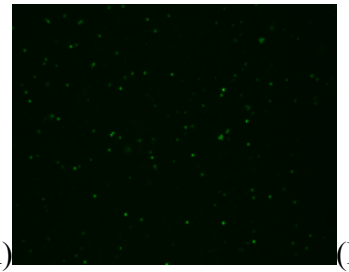
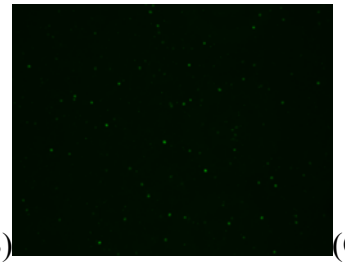
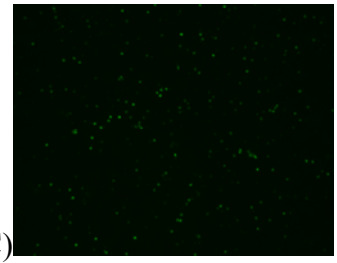
(A)  (B)  (C) 

Figure 1. Peripheral blood smear with GFP-positive cells at 1, 4 and 8 days after BMT.

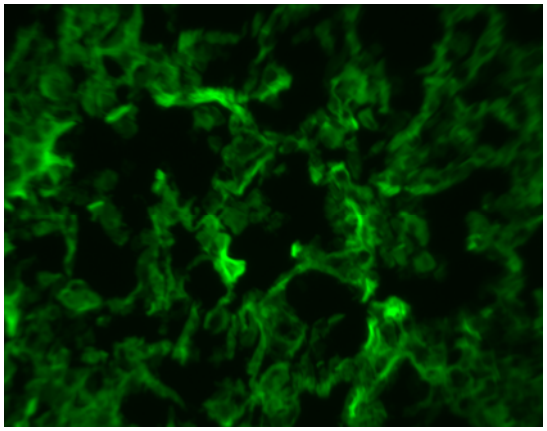


Figure 2. Lung section 1 week after induction of acute lung injury by LPS, showing positive

GFP cells in lung parenchyma.

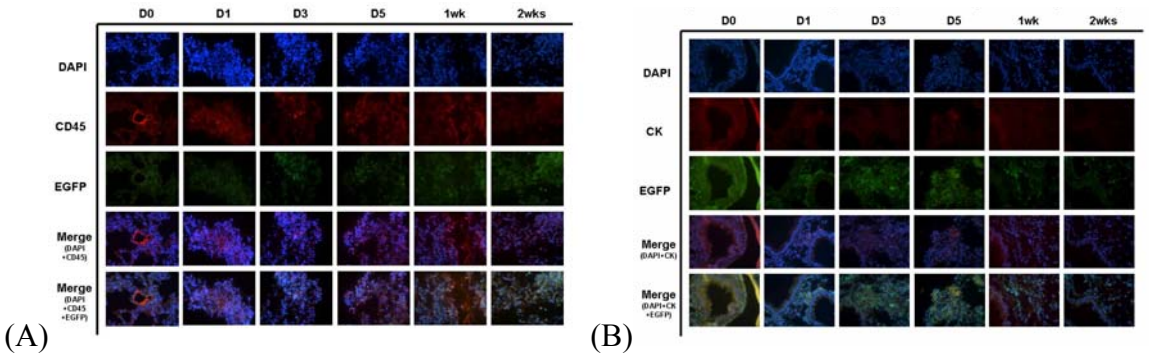


Figure 3. CD 45 (A) and cytokeratin (B) after acute lung injury

Discussion

This study has successful result in the extablishment of mice model for bone marrow transplantation. We showed good functional engraftment of GFP-positive donor bone marrow cells. By intratracheal instillation of LPS, acute lung injury developed. After that, we documented the expression of various markers, including CD 45 and cytokeratin, in the lung parenchymal cells. In the future study, further identification of source bone marrow cells, eg mesenchymal stem cells or hematopoietic stem cells, could be identified. The extent of lung inflammation, fibrosis and repair after ALI is supposed to be modified by variable treatment. Granulocyte colony stimulating factor or glucocorticoid could be tried.

Self evaluation

This study is essential for further study on the relationship between bone marrow stem cells and acute lung injury. Although we have not fully identified all the cell types in the lung tissue, the fact that the mobilization of bone marrow cells in acute lung injury has been proved.