Mouse Lung Conditioned Medium Induces Short Term Erythropoiesis in Mouse Long Term Bone Marrow Culture System.

by ***M Salimian*, M Saffari, T Khamehchian, H Nikzad, A Aliasgharzadeh.***
Research Center, Kashan University of Medical Sciences, Kashan, Iran.

Summary
Dexter-type long-term bone marrow culture is a myelopoietic culture system that allows maintenance of mouse and human hematopoiesis *in vitro* over a period of several months. In mouse unperturbed long-term bone marrow culture, erythropoiesis activity is limited to the production of immature erythroid progenitors (BFU-E) from primitive hematopoietic stem cells. In this study the effects of mouse lung conditioned medium (MLCM) as a source of myeloid growth factors, on long-term mouse bone marrow cultures was studied. Numbers of cells in adherent and non-adherent layers of cultures were counted weekly and the morphological appearances of mature cells that were produced in non-adherent layers were analyzed. In the presence of MLCM, mature nonnucleated and hemoglobinized red blood cells were produced in the non-adherent layers of the cultures.

Introduction
Long term bone marrow cultures (LTBMCs) are characterized by the formation of a complex stroma, composed of various types of stromal cells and extracellular matrix, and the maintenance of hematopoiesis *in vitro* for many weeks. In these cultures, hematopoiesis proceeds without the addition of exogenous growth factors, but is dependent on interaction between hematopoietic progenitor cells and the stroma (*Dexter, 1982; Allen et al., 1984; Eaves et al., 1991*). Stroma in human LTBMCs supports both erythropoiesis and myelopoiesis processes (*Eaves et al., 1988*). In mouse LTBMC, granulocyte and macrophage progenitors are present and undergo full development in to mature cells but differentiated erythroid progenitors (CFU-E) are not present and morphologically recognizable erythroid cells are not produced (*Oddos et al., 1987; Konno et al., 1989*). It seems that the stroma in mouse long-term bone marrow cultures does not support erythropoiesis. In this study we address the effect of Mouse lung conditioned medium (MLCM), as a source of myeloid growth factors, on the erythropoiesis process in the LTBMC system.

**Correspondence:* Morteza Salimian, Dr.
Research Center, Kashan University of Medical Sciences,
P.O.Box: 87155/111, Kashan, Iran,
E-mail: salimian_morteza@yahoo.com
Tel/Fax: +98-361-5552999

Materials and Methods
Animals
We purchased 6- to 8-week-old (18 to 22 g) BALB/c mice. These were maintained in conventional clean conditions at the animal facility of the University of Kashan, and were given mouse food and water. All animals were acclimated for at least 1 week prior to experimental use.

MLCM preparation
In order to prepare MLCM, 0.5 g of lung tissue from young BALB/c mice was incubated in 4ml of Dulbecco's modified eagle medium (DMEM, Gibco) for 48 h in fully humidified a atmosphere of 5% CO_2. The LCM was collected, centrifuged and the supernatant heated at 56ºC for 30 minute. It was then centrifuged and the supernatant was dialyzed against two changes of distilled water at 4ºC for 48h. The dialyzed LCM was centrifuged and poly-

ethylene glycol was added to a final concentration of 2% to give a clear supernatant; and filtration through 0.2 µm membrane filters sterilized the samples (*Goliaei et al., 1995*).

Bioassay of MLCM

10^5 bone marrow cells from mice were plated in 35 mm plastic petri-dishes (NUNC) containing 1ml RPMI (Gibco) supplemented with 0.3 agar and 20% Fetal Calf Serum (FCS) in the presence of 100µl MLCM. Seven days after incubation at 37ºC in a fully humidified atmosphere of 5% CO_2, the morphological identification of hematopoietic colonies and scoring were done using Wright staining. Benzidine staining was applied to demonstrate erythroid colonies (*Cooper et al., 1974*).

Long Term Bone Marrow Culture

$4x10^6$ bone marrow cells from BALB/c mice were cultured in each well of 24 well plates containing 1.5 ml DMEM medium supplemented with horse serum 25% and 10^{-7} M hydrocortisone in the presence of 50 µl of MLCM. A long term culture without MLCM was prepared as control. Cultures were incubated at 33ºC and 5% CO_2 in a humidified atmosphere. Cultures were replenished weekly by removal of 0.75 ml of supernatant and addition of 0.75 ml fresh medium. Every week the culture system was assayed for the number of cells that were produced in adherent and non-adherent layers of cultures. Giemsa staining and morphological analysis determined the various bone marrow cells in non-adherent layer of cultures.

Results and Discussion

Soft agar assay indicated that in the presence of MLCM, myeloid progenitors of bone marrow produce colonies containing mature granulocytes and macrophages but erythroid progenitors do not produce erythroid colonies (figure 1). Erythropoiesis activity in long-term bone marrow cultures in the presence of MLCM was seen. Mature nonnucleated and hemoglobinized red blood cells appeared in non-adherent layer of cultures in the presence of MLCM (figure 2). The production of mature red cells was synchronous and the erythropoiesis had a finite life. After the third week, red blood cells were not seen in the non-adherent layer of cultures. The cessation of erythropoiesis in treated cultures probably is correlated with the maturation of erythroid progenitors and no production of new erythroid progenitors from primitive hematopoietic cells. Untreated cultures did not produce red blood cells; however, they produced myeloid progenitors and mature macrophages in the non-adherent layer of the system.

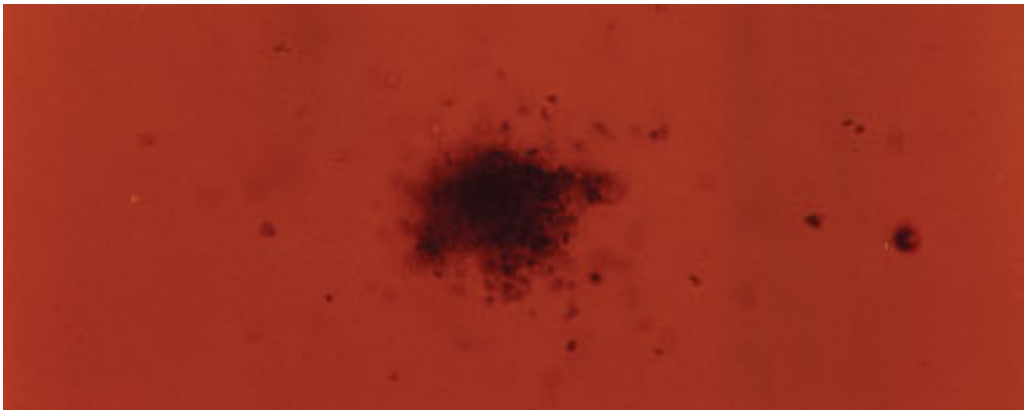

Figure1, A compact colony, containing mature granulocytes and macrophages from soft agar assay of bone marrow cells in the presence of MLCM

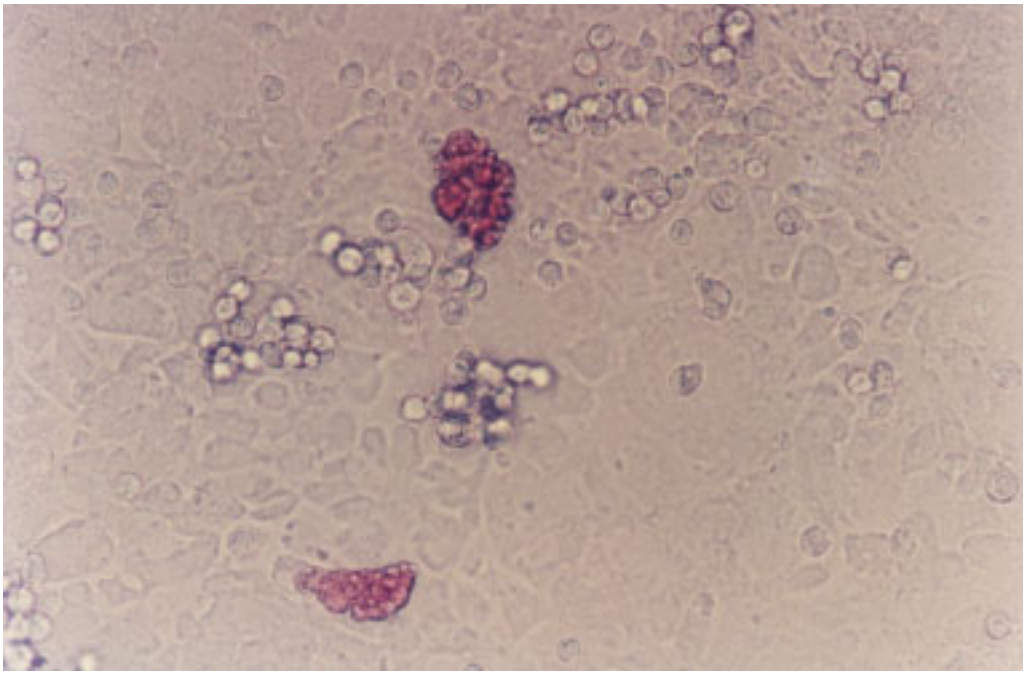

Figure2, Aggregations of nonnucleated and hemoglobinized erythrocytes are seen in non –adherent layer of long-term bone marrow culture in the presence of MLCM.

References

Allen TD & TM Dexter The essential cells of the hemopoietic microenvironment. Exp Hematol. 1984, *12*(7), 517-21.

Cooper MC, J Levy , LN Cantor, PA Marks & RA Rifkind The effect of erythropoietin on colonial growth of erythroid precursor cells in vitro. Proc Natl Acad Sci U S A. 1974, May: *71*(5): 1677-80.

Dexter TM Stromal cell associated haemopoiesis. J Cell Physiol Suppl. 1982, *1*, 87-94.

Goliaei B, Z Soheili, A Behboodi & S Samiei Hematopoiesis in the presence of macrophages in long-term bone marrow cultures. Exp Hematol. 1995, Sep; *23*(10), 1115-1120.

Eaves AC & CJ Eaves Maintenance and proliferation control of primitive hemopoietic progenitors in long-term cultures of human marrow cells. Blood Cells. 1988, *14*(2-3), 355-368.

Eaves CJ, JD Cashman, RJ Kay, GJ Dougherty, T Otsuka, LA Gaboury, DE Hogge, PM Lansdorp, AC Eaves & RK Humphries Mechanisms that regulate the cell cycle status of very primitive hematopoietic cells in long-term human marrow cultures II. Analysis of positive and negative regulators produced by stromal cells within the adherent layer. Blood, 1991. Jul 1; *78*(1): 110-117.

Konno M, C Hardy & M Tavassoli Erythropoiesis in murine long-term marrow cultures. Adv Exp Med Biol. 1989, *271*, 205-9.

Oddos T, S Nacol-Lizard & JP Blanchet Erythropoiesis in murine long-term bone-marrow cell cultures: dependence on erythropoietin and endogenous production of an erythropoietic stimulating activity. J Cell Physiol. 1987, Oct; *133*(1): 72-8.