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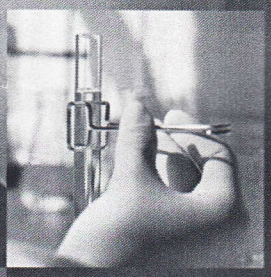

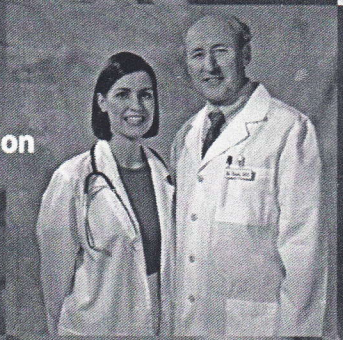

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BONE MARROW-DERIVED MACROPHAGES: ISOLATION AND CHARACTERIZATION

Danijela Živić and Snežana Živančević-Simonović
Department of Pathophysiology, Faculty of Medicine, University of Kragujevac, Serbia and Montenegro

IZOLOVANJE I KARAKTERIZACIJA MAKROFAGA KOSTNE SRŽI

Danijela Živić i Snežana Živančević-Simonović
Katedra za patološku fiziologiju, Medicinski fakultet, Univerzitet u Kragujevcu, Srbija i Crna Gora

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SAŽETAK

U ovom radu su izolovane atherentne ćelije kostne srži sa ciljem da se dobiju makrofazi kostne srži i ispita njihova morfologija i funkcija. Da bi se eliminisale dendritične ćelije, inkubacija atherentnih ćelija trajala je 24 sata. Morfologija ćelija analizirana je na preparatima koji su obojeni po metodi May-Grüenvald Giemsa, a funkcija procenjivana na osnovu ekspresije Fc receptora, sposobnosti fagocitoze čestica kvasca i sadržaja enzima nespecifične esteraze u citoplazmatskim granulama. Pokazano je da oko 95% analiziranih ćelija ima morfološke karakteristike makrofaga i da nema razlike u njihovoj funkcijskoj sposobnosti. Ovaj rezultat ukazuje na to da razlike koje postoje među makrofazima u različitim tkivima nisu posledica diferentovanja od različitih prekursorskih ćelija, već da su posledica dejstva drugih faktora, najverovatnije iz njihovog mikrookruženja.

Ključne reči: makrofazi, kostna srž, karakterizacija

ABSTRACT

In this study we analized the adherent cells isolated from bone marrow in order to investigate their morphology and function. Adherent cells were incubated for 24 hours to eliminate the transiently adherent dendritic cells. For morphology analysis cells were stained with May-Grüenvald Giemsa, and their function was estimated according to Fc receptor expression, phagocytic ability and nonspecific esterase presence in their cytoplasm. It was shown that 95% of analysed cells posseses the morphology of macrophages, as well as there was no differences in their function. These results indicate that differences between resident tissue specific macrophages were not consequence of maturation process from different cell precursors, but that these tissue specific characteristics of macrophages might be induced by some other factors, most probably from their microenvironment.

Keywords: macrophages, bone marrow, characterization

Abbrevations: EDTA- Ethilen diamino tetra acetic acid, FCS – fetal calf serum, HCl- chlorine hydrogen, MGG - May-Grüenvald Giemsa, $NaNO_2$ - Natrii nitriti, NSE – nonspecific esterase, SE – Sheep eritrocytes, PBS – Phoshate buffer salty

INTRODUCTION

Mononuclear phagocyte system is made of cells which have mutual origin, and their maturation starts by proliferation and differentiation of progenitor cells to the stage of monoblasts which with further differentiation transform to promonocytes and then to monocytes (1). Monocytes enter the circulation and stay there for about 40 hours (2) and then transfer themselves to tissues and transform to tissue macrophages (3).

Macrophages are distributed in lymphoid tissues and organs, as well as in liver, lungs, intestine, central nervous system, serous cavities, bones, synovia and skin (4). In these organs macrophages as phagocyte cells have important role in nonspecific protection of an organism (5), and as an antigen presenting cells they have a role in the process of activating specific T-lymphocytes (6). Beside that, macrophages follow physiological processes and contribute in homeostasis maintenance (7).

But although macrophages of different organs and tissues are of mutual origin (3) it is known that there are a lot of differences between them (8) as well as between macrophages placed in the same tissue (9).

Considering the fact that macrophages of bone marrow can collaborate in the presentation of proper and foreign antigens, inducing immune tolerance or immune response (10) and that bone marrow cells present common precursors of all mononuclear phagocytes (3) which have a lot of tissue specific characteristics, the aim of this study was to isolate and characterize macrophages of bone marrow.

MATERIALS AND METHODS

Isolation of bone marrow-derived macrophages

In the experiments, 6 to 8 week old male or female mice C57BL/6 were used. Mice were obtained from the Academy of Military Sciences, Belgrade. Bone marrow was isolated from femur of mice. Bone marrow was extracted by flushing the shaft from the proximal side with 1ml PBS solution. The cells of bone marrow were washed twice in the PBS solution by centrifugation at 200xg for 10 minutes (4°C). Cells were suspended in RPMI 1640 medium (Gibco) containing 100 IU/ml of penicillin, 100μg/ml of streptomycin, 2 mM L-glutamine and 10% fetal calf serum, FCS, Gibco). Macrophages were isolated from nonadherent cells of bone marrow by adherence on plastic surface, in 100 mm Petri dishes (Falcon) by incubation at 37°C in 5% CO_2 in a humidified atmosphere incubator (Heraus). After 24 hours of incubation, the monolayer was washed with PBS in order to remove non-adherent cells and adherent cells were removed from the plastic surface by method of Chu et al. (11). Petri dishes, with 5ml of cold PBS solution containing 0.02% disodium EDTA, were incubated at 4°C for 20 minutes and then cells were partially removed from the surface by squirting the solution on the dishes vigorously. Remaining adherent cells were scraped off with a rubber policeman. Cells were washed three times

Correspondence:
Danijela Živić
School of Medicine, University of Kragujevac,
Svetozara Markovića 69, 34000 Kragujevac, Serbia and Montenegro
Tel: + +381 34 335 572, Fax: + +381 34 306 800