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RAT MICROGLIA CELLS:THEIR CULTURE, ISOLATION AND PHAGOCYTIC ACTIVITY

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Abstract

Microglia were isolated from mixed primary cell cultures of the cerebral cortex from 3day old male Wistar rats. The mechanically dissociated cells were plated in a flask at a density of 10^7per 300 cm^2 and maintained at 37°C in a 10 % CO_2/90 % air atmosphere. After 10-14 days in culture, floating and weakly attached cells on the mixed primary cultured cell layer were isolated by gentle shaking of the flask for 3-5 min. The resulting cell suspension was transferred to plastic dishes and allowed to adhere at 37°C. To investigate the morphological change of microglia, the cells after 2 days of culture were incubated with biotinylated GSA-I-B4 (10μg/ml) at 4°C for overnight. To detect the phagocytic activity, isolated microglia were incubated with opsonized zymosan (20 mgl/ml) for Ih at 37°C and with Giemsa's staining solution for 30 min at room temperature. The results were about 90 % of attached cells were positive for OX6. Morphologically, most of the isolated microglial cells had amoeboid and rod-shaped cell bodies with no or a few thick processes. Most of these cells became amoeboid-like cells and showed a number of vacuoles in the cytosol when cultured in the presence of IFN-γ+ LPS. Both control and IFN-γ + LPS - treated cells exhibited the intense phagocytic activity against zymosan particles.

Keywords: Microglia cells, Primary cell cultures, Phagocytic activity

Introduction

Microglia form a regularly spaced network of resident glial cells throughout the central nervous system (CNS). They are morphologically, immunophenotypically and functionally related to cells of the monocyte/macrophage lineage[1]. Microglia are believed to be important in modulating neuronal function and to have macrophage- like function in the central nervous system[2]. As microglia as well as other phagocytic cells can be primed (preactivation) by exposure to a variety of agents including LPS, platelet activating factor (PAF), diacylglycerol. Some major cytokines, like IFN γ , IL-1, IL-2, IL-4, TNF-α have the ability to prime phagocytic cells [3,4,5]. The development of techniques for the isolation and maintenance of microglia cells in culture has provided researchers with a valuable experimental system for investigating of microglial cell function[6]. The use of isolated microgia cells to

study various aspects of cathepsin E have been reviewed elsewhere [7,8,9]. In this paper we describe techniques for culture, isolation and phagocytic activity of microglia.

Materials and Methods

1. Materials

3-day old male Wistar rats, Ca^{2+} and Mg^{2+}- free phosphate buffered saline (CMF-PBS) (Gibco, Gaithersburg,MD), papain and DNase (Worthington, freehold, NJ), D,L-cysteine-HCL, bovine serum albumine, glucose, fetal calf serum (FCS), Dulbecco's modified Eagle's medium (DMEM), $NaHCO_3$, penicillin-streptomycin, cell stainer with 70 Jlm pore size (Falcon, Franklin Lakes, NJ), antibodies recognizing microglia (OX6) and glial fibrillary acidic protein (GFAP) were purchased from Serotec and DAKO respectively, flask and chamber slides (Nunc Inc.,Naperville, IL), opsonized zymosan, biotinylated GSA-I-B4. Avidin-biotin-peroxidase complex (ABC) (Vectastain Kit, vector Lab., Burlingame,CA), 3',3- diamilloben-zidine (DAB). Zymosan A (20mg/ml) was opsonized with fresh serum and used either immediately or stored at 70°C.

2. Cell Culture

Microglia were isolated from mixed primary cell cultures of the cerebral cortex from 3-day old male Wistar rats according to the modification method described by Nakajima et al. (1992). The cerebral cortex was dissected, freed of meninges ami blood vessels, soaked in Ca^{2+} and Mg^{2+} - free phosphate buffered saline (CMF-PBS) cuid minced with a razor blade. Then, the tissue was enzymatically dissociated by incubation twice for 15 min at 37°C IN CMF-PBS containing papain (90 units/ml), DNase (2000 units/ml),d,l-cysteine-HCL (2.23 mg/ml), bovine serum albumin (2 mg/ml) and glucose (50 mg/ml). After termination of the reaction by adding fetal calf serum (FCS), the tissue fragments were isolated by centrifugation and resuspended in the culture medium consisting of Dulbecco's modified Eagle's medium (DMEM), 0.3% $NaHCO_3$, 50 units/ml penicillin, 100 µg/ml streptomycin, and 10 % FCS. The tissue fragments were then dissociated by gentle passage through plastic tips with three different diameters. The mechanically dissociated cells were filtered through a cell strainer with *70µm* pore size. Cell were plated in a flask at a density of 107per 300 cm^2 and maintained at 37°C in a 10 % CO_2/90 % air atmosphere. Subsequent medium replacement was carried out on every 3 days.

3. Immunocytochemistry

To investigate the morphological change of microglia, the cells after 2 days of culture were washed with PBS several time and incubated with biotinylated GSA-I-B4 (10µg/ml) at 4°C for overnight. After washing with PBS, the cells were processed with ABC method and visualized with DAB. To detect the phagocytic activity, isolated microglia were transferred to the chamber slides, unattached cells were removed after 30 min and strongly attached microglia were resuspended in the culture medium consisting DMEM, 0,3% NaHCO3, 50 units/ml penicillin, 100µg/ml streptomycin and 10 % FCS. Then IFN-γ 100U and LPS lµg/ml were added as activator of microglia. The culture was maintained at 37°C in a 10 % CO_2/90 % air atmosphere. After 2 days in culture, the cells were incubated with opsonized zymosan (20 mg/ml) for 1h at 37°C. After several washes with PBS, the cells were incubated with Giemsa's staining solution for 30 min at room temperature and observed with a bright-field microscope[10].

Results

1. Isolate glia cells

After 10-14 days in culture, floating and weakly attached cells on the mixed primary cultured cell layer were isolated by gentle shaking of the flask for 3-5 min. The resulting cell suspension was transferred to plastic dishes and allowed to adhere at 37°C. Unattached cells were removed after 30 min., microglia were isolated as strongly adhering cells. About 90 % of these attached cells were positive for OX6, a marker for macrophage/ microglial cell types (Fig. 1A). After isolation of microglia from the mixed cell culture, Oligodendrocytes were removed by vigorously shaking with a shaker (BR-40LF, TAITEC, Japan) with 160-200 stroke/min for 16 h at 37°C. About 95 % of the remaining cells were positive for antibodies to GFAP, a marker for astrocytes (Fig.1B).

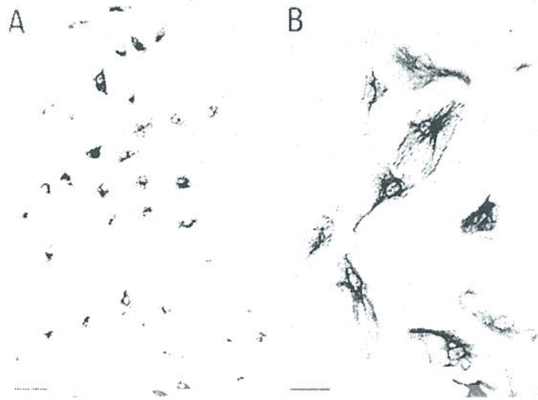

Fig. 1 Immunohistochemical staining of microglia (A) and astrocytes (B) isolated from the cerebral cortex from 3-day old rats. Microglia and astrocytes were stained with OX6 and anti-GFAP antibody, respectively. Bars = *20μm*.

2. Changes in Morphological and Phagocytic Activity

Morphologically, most of the isolated microglial cells had amoeboid and rod-shaped cell bodies with no or a few thick processes (Fig. 2A). Most of these cells became amoeboid-like cells and showed a number of vacuoles in the cytosol when cultured in the presence of IFN-γ + LPS (Fig 2B). Both control and IFN-γ+ LPS - treated cells exhibited the intense phagocytic activity against opsonized zymosan particles (Fig. 2 C, D). There was significant difference in the number of the particles taken up between control and IFN-γ + LPS.

Fig. 2. Morphological change and phagocytic activity of cultured rat microglia. Isolated microglia were cultured in the absence (A) or presence of IFN-γ + LPS for 2 days and then stained with B-4 Isolectin. Note that control cells (A) showed rod-or amoeboid-shaped cell bodies, but most of the IFN-γ + LPS treated cells became amoeboid like cells (B). Both control (C) and IFN-γ + LPS treated cells (D) showed a similar phagocytic activity against zymosan particles. Bar = 20 μm.

Discussion

Since microglia were discovered and described as a separate cell type of the brain, microglial research was neglected until recently. The introduction of microglial cell cultures [1,6] opened possibilities for studying more detailed functions of this cell type. Two principal cell types were identified the amoeboid cells also referred to reactive migroglia and the ramified cells[11]. Microglia functions in particular as brain macrophages, antigen presenting cell and immunoeffector cell involved in local inflammatory responses of the brain [2,10,12,12]. In this report we have described successful isolation and cultivation of microglia cells which were investigated for their morphology, responses to IFN-γ + LPS and their functional properties.

Microglia cells which were isolated from primary glia cells cultures of 3 days rat. By morphologic analysis, most of the isolated microglial cells had amoeboid and rod-shaped cell bodies with no or a few thick processes (Fig. 2A). Most of the cultured cells were identified as microglial cells as they were positive for monoclonal antibodies (Sera Lab) OX6 (Fig. 2A). OX6 which is a marker for amoeboid microglial cells (an agent known to recognize MHC class II).

Microglia can be activated by various internal and external stimuli or are extremely sensitive towards any kind of stimulus. Using analogous method to establish microglial cell cultures it has been observed recently that rod-shaped cell bodies with no or a few thick processes transform into amoeboid cells when exposed to IFN-γ + LPS (Fig. 1 B). The modes of culturing which were used to induce certain characteristics of differentiated cells have also been employed be several other authors. For example, treatment with IFN-γ microglial cells became Ia positive and functioned as antigen-presenting cells. Futhermore microglial cells exposed to IFN-γ + LPS developed tumor cell cytotoxicity and produce tumor necrosis factor α . Taken together microglial cells share the characteristic of cells of the macrophage lineage[12] . IFN-γ + LPS were used to make sure that the effector cells remained fully activated.

We show that functional characterization of rat microglial cells. Both control and IFN-γ + LPS treated cells exhibited the intense phagocytic activity

against zymosan particles (Fig. 2 C,D). Activation of the microglial cells with IFN-γ + LPS resulted in significant increase in phagocytosis than untreated microglial cells. In term of functional changes, activated microglia may release several mediator substances including cytotoxic compound ie. Reactive oxygen intermediates, nitric oxide or protease and inflammatory cytokines. LPS or IFN-γ resulted in strong up-regulation of proinflamatory cytokines [2,13]. More over, microglia activation take place in grade fashion, in a fist step of activation, microglia become activated as evidenced by the criteria mentioned above, but do not phagocytic. In a second step of activation, activated microglia futher transform into phagocytic cells[2]. The Conclusion is microglial cells culture can be used as a model for studying more detailed functions of this cell type.

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