LONG TERM CULTURE OF BOVINE PULMONARY ALVEOLAR MACROPHAGES IN HOLLOW FIBER CAPILLARY

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Introduction

Macrophages play an important role in resistance to diseases by phagocytizing and killing the bacteria, or by secreting various active substances. However, most such information comes from work with macrophages of humans, mice and so on. Little information is available whether the macrophages from cattle secrete active substances. One of the reasons is that the culture of bovine macrophages is limited to a few days during their incubation and it is very difficult to obtain a sufficient yield of the products to determine the physical properties of their biological functions.

Therefore, in these experiments, the first and probably the most important step is the establishment of the culture system. It is the purpose in the present report to cultivate the macrophages from bovine pulmonary alveoles in culture flasks and hollow fiber capillary over a long term.

Materials and Methods

Cell culture of bovine alveolar macrophages

Bovine alveolar macrophages were collected by lung washing of slaghtered animals as previously described (Inooka, 1988). In general, the cells were cultivated in 25 cm^2 culture flasks and Dulbecco's modified Eagles' medium containing 10% fetal calf serum (D-MEM). After 2-24 hours of incubation at 37°C in CO_2 of 5%, unadherent cells were washed and new D-MEM were added and continued in culture. The cell activities were mainly observed under phase microscopy.

Effect of mitogen on cell growth and morphology

Bovine alveolar macrophages (10^5) were inoculated in 24 well mult plates and cultivated for 2 hours. After washing, 0.15 μl of pytohemagllutinin M (5 ml; Bacto) was added. Cell growth and morphology were observed under phase microscopy.

Cell culture in hollow fiber capillary

A Hollow fiber bioreactor (figure 1) was experimentally constructed using Minivita fiber (30,000 MW cut off, Grade Japan) (figure 1A). Bioreactor systems were operated at 37°C, 5% CO_2 and pH 7.0. Medium, containing 500 ml medium in reservoir (figure 1B), was recirculated at a rate of 20 ml/min. During incubation, both fluids from the extracapillary space and recirculating medium were harvested and mainly analyzed according to the number and viability of the cells which were detached from fiber, and glucose consumption.

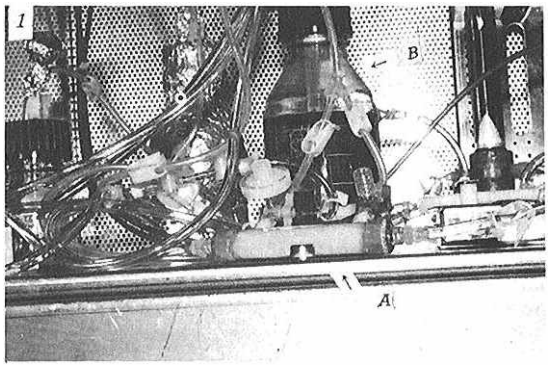

Figure 1. Hollow fiber bioreactor which is experimentally constructed. Minivita fiber (A) Reservior of medium (B).

Results

Cell culture of bovine alveolar macrophages in culture flasks

Bovine alveolar macrophages were isolated and

cultivated in D-MEM for 24 hours. After the removal of nonadherent cells, the medium was exchanged and culture was continued. Most of the adherent cells were macrophages based on morphology.

About half of these cells were round in shape and the remainder spread. At times, fibroblast-like cells were grown. In these conditions, the macrophages were maintained in culture for up to 50 days. Glucose consumption was 1.4 mg/10^6 cells/day until 5 days of culture. After this, the cells were maintained without remarkable glucose consumption.

Effect of mitogen on cell and morphology

Remarkable changes in the shape were observed when PHA was added to macrophages. The individual cells are distinctly longer and more fibroblast-like as compared to the macrophages grown in the absence of PHA.

Cell culture in hollow fiber capillary

In the first experiments, macrophages were cultured for 30 days in flasks. Macrophages were trypsinized. The Hollow fiber bioreactor was operated in D-MEM for inoculation with 1.8 x 10^6 cells. The macrophages were maintained in culture up to 25 days without cell death detached with a small amount of glucose consumption.

In the second experiment, macrophages after culture for 2 days were trypsinized. Medium (serum-free medium) was recirculated and 1.0 x 10^6 of cells were inoculated. After 2 hours, PHA (270 μl) was added to the extracapillary. About 1/10 of macrophages died after 2 days and glucose consumption (10 mg/10^6/day) was detected in 2 days of culture. After this, the cells were maintained without cell death or remarkable glucose consumption.

Discussion

At present, macrophages can not be divided in normal state. Therefore, a macrophage cell line must be established or a large number of macrophages must be collected from cattle for obtaining cell products from macrophages. In this experiment, we used the alveolar macrophages, because the cells could be collected in large numbers of homologenous macrophages (over 10^9/a head), and very easily. This suggested that macrophages in the lungs is available to obtain a sufficient yield of cell products.

Here, we found that the macrophages were maintained in culture up to 50 days in two shapes; namely a rounded shape without PHA and fibroblast-like cells with PHA. Moreover, macrophages which were morphologically transformed by the addition of PHA, could be cultivated in serum-free medium for long term (Data was not shown). This suggested that the large number of macrophages from bovines could be cultivated a long time in a serum free medium.

On the other hand, cell cultures which used hollow fiber, have been often employed for obtaining monoclonal antibody and so on. However, it is unclear whether macrophages were cultivated by hollow fiber, because macrophages have strong adherent activity to fiber. In this experiment, we showed that bovine macrophages were continuously cultivated by the method of hollow fiber culture.

Although the analysis of cell products is in progress, the present report shows that this culture system of bovine macrophages provides a tool to resolve a cell substance from bovine macrophages or to obtain interferon, vaccin and so on which is produced by the use of macrophages.

(Key Words: Bovine Alveolar Macrophages, Hollow Fiber Culture, PHA)

Literature Cited

Inooka, S. 1988. Blood monocytes cell lines from cattle and several species: including collection of bovine alveolar macrophages. Communications in Applied Cell Biology 6(1-2): 10-13.