SOME BIOLOGICALLY ACTIVE SUBSTANCES PRODUCED BY THE BOVINE RUMEN MUCOSA CELLS AND ALVEOLAR MACROPHAGES IN CULTURE

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Introduction

Ruminants absorb and metabolize volatile fatty acids in the rumen mucosa epithelium and use these substances for energy source. However, most information comes from work with pieces of tissues. This is because cell culture techniques for this tissue are not avilable for in vitro sudies. Therefore, there is no evidence as to whether or not there is an interaction between cells in the growth of the rumen epithelium. Moreover, it is unclear whether the cells which contain heterogenous complex cells, mainly of corticated, granulosum, spinosum, basal cells and the migrated cells such as macrophages, have a role in controlling mucosal disease such as viral diarrhea.

We here describe that the rumen mucosa cells and alveolar macrophages produced some active substances.

Materials and Methods

Cell culture of bovine rumen mucosa tissue and alveolar macrophages

Cell separation and culture from rumen paillae in the bovine caudal blind sac was carried out in 25 cm^2 flasks as described previously (Inooka, 1987). After 14-20 days of cultures (sprit ratio: about 1/4), culture fluids (RMCF) were collected, and used to assay bioactive substances after sterilization by milliporefilter.

Bovine alveolar macrophages were collected by lung washing of slaughtered animals as previously described (Inooka, 1988). After 20-30 days of cultures, the culture fluids (BPMC) were collected and used for assay.

Morphological changes of rumen mucosa cells in RMCF

The cells were separated from rumen mucosa tissues. These cells were suspended in the RMCF and cultivated in 24 well plates at 37°C for 30 minutes. The cell morphology was observed under phase microscopy.

Assay of endothelial cell growth factor umbilical veins (ECGF) in RMCF and BPMC

Human endothelial cells from the umbilical veins (5×10^3) were incubated in a 24 well plates and the 199 medium contained 2.5% calf fetal serum in control culture, and the 199 medium plus 25% RMCF and BPMC in experimental culture. After 3 days of incubation, the number of cells was counted. The rate of growth was calculated by the number of cells in the control culture to the cell number in the experimental culture.

Influence of 7TD1 cells growth in the RMCF and BPMC

7TD1 cells (mouse hybridoma tumor cells), which the growth is dependent on Interleukin-6, were cultivated in 96 well plates. The growth assay was determined by the Messmann method (J. Immunol. Methods. 65, 55-63, 1983).

Results and Discussion

Morphological changes of rumen mucosa cells in RMCF

In a previous study (Inooka, 1987), we have reported that four types of cells were separated from rumen tissues and two types of cells, epithelial and fibroblast-like cells, could be cultivated from these cells. Morphological changes in small round cells (probably spinosum cells) were observed when the RMCF (most of which grew were fibroblast-shaped cells) was added. The cells were distinctly longer or under division.

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This finding showed that some cytotoxic factors to rumen mucosa cells were contained in RMCF, and suggested that the cells were easily changeable in morphology.

BCGF activity in the RMCF

Growth enhancement of HEC was observed when RMCF (most of which grew were the epithelial cells in a small colony) was added (growth rate 1.27). No growth enhancement was observed when other RMCF and BPMF were added. This suggested that the factors, produced from rumen epithelial cells, influenced the growth of the other cells in rumen tissues. We are studying cell cloning to produce ECGF and to clarify the characteristics of ECGF.

Growth inhibition of 7TD1 cells in the BPMC

Growth inhibition of 7TD1 cells was observed when BPMC was added (table 1). Growth inhibition of 50% cells was dilutions of 32 times of BPMC. No growth inhibition was observed when the culture fluids from rumen mucosa cell culture (RMCF) were added.

TABLE 1. GROWTH INHIBITION OF 7TD1 CELLS IN THE BOVINE ALVEOLAR MACROPHAGE CULTURE FLUIDS (%*)

The culture fluids	Dilution of culture fluids (x)							
	6	12	24	48	96	192	384	768
Bovine alveolar macrophages (1) (10 days of culture)	33.3	36.6	41.5	45.9	71.4	66.7	77.8	87.5
Bovine alveolar macrophages (2) (10 days of culture)	28.6	31.7	29.3	40.5	53.6	61.9	61.1	62.5
Rumen mucosal cells (1) (14 days of culture)	81.0	112.3	104.9	100.0	100.0	95.2	105.6	104.2
Rumen mucosal cells (2) (14 days of culture)	71.4	112.2	97.6	91.9	82.1	71.4	–	66.7
D-MEM**	76.2	95.1	100.0	89.2	103.6	114.3	72.2	91.7
Interleukin-6**	471.4	251.2	268.3	275.7	264.3	238.1	138.9	104.2

$$* \% = \frac{\text{The number of cells in the culture fluids}}{\text{The number of cells in the control culture medium}} \times 100$$

** D-MEM which was used in cell culture, and Interleukin-6 were added instead of the culture fluids.

The facts show that the macrophages produced the cytotoxic substances for tumor cells and did not influence the normal cells, whereas rumen mucosa cells produced the growth factors of normal cells and did not influence the tumor cells.

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(Key Words: Rumen Mucosal Cell, Growth Factor, 7TD1 Cells)

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