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Inhibition of Growth of Measles Virus by Mycoplasma in Cell-cultures and the Restoring Effect of Arginine

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Summary

Presence of mycoplasma in MK cell cultures caused an inhibition of measles virus, as revealed by a lower plating efficiency. When arginine is supplemented to the cell-system, mycoplasma contaminated cells yield plaque titers as high as those of uncontaminated cells. The essential role of arginine for plaque formation of measles virus is suggested also by experiments in which arginine added to the agar overlay restored the plating efficiency of measles virus in cultures maintained in a minimal nutrient medium.

1. Introduction

Presence of mycoplasma has been often reported in primary or continuous cell lines by several authors. Contaminated cells exhibit sometimes morphological changes and growth rate alteration. Inhibition of virus multiplication in mycoplasma infected tissue cultures has been also described (1, 2, 3).

Rouse, Bonifas, and Schlesinger (1963) observed that KB cell cultures contaminated by mycoplasma were unable to support the growth of adenoviruses; this condition was reversed by supplementing the medium with arginine or by suppressing mycoplasma growth with kanamycin. As mycoplasmas actively degrade arginine, it was concluded that this amino acid is essential for adenovirus biosynthesis.

The present investigation was undertaken to determine if mycoplasma produced depletion of arginine in primary cell cultures interferes with measles virus growth, as assayed by plaque formation.

2. Materials and Methods

2.1. Cultivation of Mycoplasma

M. orale, strain Symons, was kindly supplied by K. Lind, Statens Seruminstitut, Copenhagen. Media used for the growth and maintenance were prepared according to the method described by Chanock and Hayflick (1962).

2.2. Tissue Culture

Kidney cell monolayers from African green monkey (MK) *(Cercopithecus aethiops sabaeus)* prepared by *Youngner*'s method (1954) were used throughout. Growth medium

consisted of 0.5% lactalbumin hydrolysate in Hanks' BSS supplemented with 0.2% yeast extract and 2.5% calf serum. Cultures were maintained in 0.5% lactalbumin hydrolysate in Earle's BSS with 0.2% yeast extract.

2.3. Virus

The local strain Biseci of measles virus, grown in monkey kidney tissue culture (4) was used as well as the Edmonston strain.

2.4. Plaque Count of Measles Virus

Fully grown monolayers of MK in rubber stoppered 3 oz. prescription bottles were used. Just before use, nutrient fluid was removed and the cell sheets were washed once in warm (37°C) phosphate buffered saline (PBS). Cell sheets were infected with 0.2 ml of an appropriate dilution of the strain Biseci, allowed to adsorb for 1 hour at room temperature with occasional gentle rocking of the plates. At the end of the adsorption period, 12 ml of overlay agar (1.5%) in Earle's salt solution (ESS), containing 2% calf serum and 0.0017% neutral red were added to each bottle which were then incubated at 37°C. Some experiments (see Results) were performed with agar ESS enriched with arginine to a final concentration of 0.5 mM per liter.

Table 1. *Measles Virus Plaque Counts in Mycoplasma Preinfected and Uninfected Cultures*

Cultures	Measles virus (PFU/0.2 ml)[1]
Uninfected	640
Myco. infected on day 0	50
Myco. infected on day 2	80
Myco. infected on day 5	500

[1] Infection with measles virus at the 6th day after cell seeding.

3. Results

A preliminary experiment consisted in determining the pattern of growth of mycoplasma in MK cell cultures.

0.2 ml amounts of broth culture of *M. orale* were added to the fluid medium of 30 bottles seeded with 8 ml of trypsinized cell suspension at the time of seeding or 2 and 5 days thereafter. Control bottles received the same amount of uninfected broth. The infected tissue cultures did not exhibit any cytopathic effect or change of pH over a period of 12 days of observation. Titration of the supernatant showed a continuous increase in colony forming units (CFU) titre of *M. orale* during the first 6 days, followed by a decrease in colony number until colonies could not be obtained on plates inoculated with undiluted fluid at the 10th day.

Six days after the cell seeding, all groups of mycoplasma preinfected bottles and uninfected bottles were inoculated with 100—1000 PFU of measles virus (strain Biseci) in 0.2 ml. The growth of measles virus was estimated by the plaque count method.

The results are shown in Table 1. It can be seen that the number of plaques formed by measles virus in the mycoplasma infected cultures is far less than that in the control cultures. The reduction in plaque numbers is more drastic if the time allowed for establishment of mycoplasma infection in cell cultures is longer (6, 4,

1 days, respectively). A possible explanation of the results was considered on the basis of SMITH's finding (1960) that mycoplasmas actively degrade arginine.

Experiments were then designed to verify if the inhibition of growth of measles virus in mycoplasma infected cell cultures was reversed by supplementing the medium with arginine.

The experiment reported in Table 2 shows that the addition of arginine to the medium effectively restores the capacity of measles virus to grow in mycoplasma-infected cultures.

Arginine Requirement for Measles Virus Plaque Production

The results of the experiment already described (Table 2), lead us to investigate the influence of arginine on the growth of measles virus, as evidenced by its capacity to restore plaque formation in cell cultures. Therefore, to exhaust endogenous reserve of amino acids fully grown monolayers were incubated for 48 hours in a balanced salt solution containing only glucose and glutamine. 100—1000 PFU

Table 2. *Measles Virus Plaque Counts in Mycoplasma Preinfected and Uninfected Cultures* (agar-overlay with arginine supplement)

Cultures	Measles virus (PFU/0.2 ml)[1]
Uninfected	620
Myco. infected on day 0	500
Myco. infected on day 2	500
Myco. infected on day 5	650

[1] Infection with measles virus at the 6th day after cell seeding.

Table 3. *Plaque Formation of Measles Virus in Cells Maintained in BSS-glucose-glutamine (BSS-GG) Medium*

Cultures[1]	Agar overlay	
	Normal	+ Arginine
Control[2]	400[4]	500
BSS+GG[3]	100	550

[1] Incubated for 48 hours.
[2] Complete medium.
[3] Balanced salt solution with glucose and glutamine.
[4] Number of plaques.

of measles virus were inoculated into the cell cultures. After adsorption for 1 hour, the cells were washed to remove unadsorbed virus. Agar overlay with and without 0.5 mM of arginine was then added on the cell sheet. The results are presented in Table 3, which shows that the reduction of plaque formation in arginine depleted cell cultures is only partial ($^1/_4$ of the number formed in cultures maintained in complete medium). This might be due to the presence of uncompletely exhausted arginine of intracellular origin or to the arginine content of the serum contained in the agar overlay. Nevertheless, the effect of arginine in restoring the ability of measles virus to form plaques seems to be substantiated.

4. Discussion

Mycoplasmas in cell cultures can influence the replication of viruses, as discussed by BROWNSTEIN and GRAHAM (1) and ARMSTRONG, HENLE, SOMERSON, and HAYFLICK (2). CANTELL and PAUKER (3) suggested that myoplasmas may stimulate interferon production in infected cultures. However, the hypothesis that viral inhibition is due to the effects induced by mycoplamas on cell metabolism (7) is more widely accepted. Although the nature of the cellular metabolic pathways

influenced by mycoplasma infection is largely unknown, in at least one case contamination with such organisms was shown to produce a specific effect on cellular metabolism. ROUSE, BONIFAS, and SCHLESINGER (5) demonstrated that the inability of adenovirus to grow in mycoplasma infected KB cells is due to the depletion of arginine caused by metabolic attack of mycoplasma on this amino acid. As shown by SCHIMCKE (8) growth of the non-fermenting mycoplasmas is dependent on the arginine present in the medium through the so called "arginine dehydrolase pathway". At present this "pathway" is accepted as the major energy source of the non-fermenting mycoplasmas.

Two main conclusions can be drawn from our investigations:

a) arginine restores the ability of measles virus to grow in mycoplasma infected cells, as assayed by plaque formation and,

b) arginine depleted cells are less able than normally fed cells to support the growth of measles virus; this effect is reversed by arginine supplementation.

As already observed, the residual ability of depleted cells to support a reduced growth of measles virus could be attributed to an uncomplete exhaustion of arginine content of the cells or to the presence of arginine in the serum of the agar overlay. Further investigations are needed to elucidate this point. The possibility that a virus variant exists being not dependent on the presence of extracellular arginine should be also taken into consideration. In any case, it is suggested that arginine plays a role in the synthesis of measles virus, a condition already described for the growth cycle of adenoviruses. Besides adenoviruses, other viruses in cell cultures, such as poliovirus (9) and herpes simplex virus (10) show a similar arginine requirement. Changes in arginase activity and arginine utilization have also been observed in cells infected with Bunyamwera (11) and with rabbit papilloma viruses (12). However, further experiments are needed to identify the step of the biosynthetic pathway of measles virus hampered by arginine depletion.

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