Effect of Mycoplasmas on Virus Replication and Plaque Formation in Mouse Cells (36075)

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Because of the high incidence of mycoplasma contamination in cell cultures we have become interested in exploring the effects of these organisms on virus multiplication and plaque formation. Since it is now apparent that either significantly increased (1–3) or decreased (3–7) yields of virus are possible from mycoplasma-infected cells we have attempted to determine what role the type of cells used in the experiments plays in these interactions. Accordingly, we compared primary mouse embryo fibroblast (MEF) cultures with L cells, a continuous mouse cell strain.

Materials and Methods. MEF monolayers were prepared in 2 oz bottles by the Tissue Culture Section, Laboratory of Virology and Rickettsiology, DBS, from 15–17 day old embryos, and the L cells were kindly supplied by the Laboratory of Viral Diseases, NIAID. All cell cultures were maintained in Eagle's minimal essential medium (MEME) containing 4.8 mmoles of glutamine to which was added 1% fetal calf serum. The latter was obtained commercially, and filtered before use in a Nalgene filter unit (10^{-2} μ plain membrane) since filtration was found to reduce the risk of bovine mycoplasma contamination of cell cultures. Antibiotics were omitted from the media. Only cultures which were initially free of contaminating mycoplasma were used.

Mycoplasma arginini, strain G230, and *Mycoplasma hyorhinis,* strain 545, have been described elsewhere (2). Pools were prepared in broth, and cell cultures were inoculated with at least 10 colony forming units of *M. hyorhinis* or *M. arginini*. Chronic infection was readily established. Superinfection with a virus occurred on day 3 post mycoplasma inoculation at a time when there were no observable differences in viable cell counts between mycoplasma infected and mycoplasma-free cells. MEF cultures inoculated with *M. hyorhinis* exhibited increased acidity at this time, but no observable cytopathic effects were noted during the study period.

Semliki Forest virus (SFV) and a large plaque variant of vesicular stomatitis virus (VSV), Indiana strain, were prepared in chick embryo fibroblast cell cultures (CEF). Details of the plaquing technique have been reported elsewhere (2).

Each monolayer was challenged with 30–100 plaque forming units (PFU) of either SFV or VSV. This very low input of virus was chosen since previous experiments in another system (1) had indicated that a low input of virus with subsequent multiple growth cycles was the most sensitive way of detecting the effects of mycoplasma on virus replication. In virus replication experiments, supernatants were collected 18–24 hr postvirus inoculation and then assayed for virus content, and an increase or decrease in virus yields of 0.5 $\log_{10}$ or greater was considered to be significant ($p \geqq .05$).

Results. Effect on virus yields. As shown in Table I, results from cultures infected with *M. arginini* and virus differed in MEF and L cells. With VSV, enhanced yields were obtained in MEF but not in L cells. With SFV, enhanced yields (3 out of 4 experiments) were obtained in L cells but not in MEF. With *M. hyorhinis,* results also differed somewhat in the two systems. Although decreased yields of VSV were obtained in both MEF and L cells with SFV, decreased yields were observed in MEF but increased yields were seen in L cells. Thus, the same virus and

TABLE I. Differences in Virus Yields from Mycoplasma-Free and Mycoplasma-Infected Mouse Cells.

Cell cultures chronically infected with:	VSV	SFV
	Primary mouse embryo fibroblasts	
M. arginini	+.8,[a] +.7, +.9, +.6, ND[b] +1.0	(±0),[c] (+.1), (—.2), ND
M. hyorhinis	—.8, —.9, —.5, —.6, —1.0, —.9	—.8, —.7, —.8, —.5
	L cells	
M. arginini	(—.1), (—.2), (±0), (—.1)	(+.4), +1.6, +1.1
M. hyorhinis	—1.3, —.8, (—.1), —.7	+.6, +1.7, +1.1

[a] Values expressed as $\log_{10}$ difference from control (mycoplasma-free) cells.
[b] ND = not done.
[c] ( ) = result not statistically significant at the $p < .05$ level.

mycoplasma interacted in a different manner in the different cell systems.

Effect on plaque morphology and number. Differences in plaque morphology and number were also noted when these viruses were plaqued directly onto mycoplasma-infected cells. In MEF, plaque counts in mycoplasma-infected cells were always less than in mycoplasma-free cells (Table II). In addition, there was a marked decrease in the size of the plaques formed by both viruses in the mycoplasma-infected cultures. This change in plaque morphology was most striking in cultures infected with SFV (Fig. 1), in which, after 3 days of incubation, there was a marked reduction in the size of the SFV plaques in *M. hyorhinis*- or *M. arginini*-infected cultures.

Simultaneous inoculation of the virus and either mycoplasma in MEF had no effect on virus plaque count or morphology, indicating that preinfection with the mycoplasma was a necessary prerequisite for this phenomenon. In addition, interferon was not detected at any time in the supernatants of mycoplasma-infected MEF cells. In L cells, plaques of VSV and SFV developed with equal efficiency in mycoplasma-free and infected cells and neither plaque size nor count differed appreciably (Table II).

Discussion. The observation of altered virus plaque morphology in the presence of mycoplasma has been seen previously (4) and could be of importance in studies where plaque size serves as a marker. Changing plaque morphology may not be a function of the virus but may simply reflect mycoplasma contamination of the assay system.

The differences in virus yields in the above *in vitro* experiments, have generally been in the range of 0.5 to 1.7 logs. It is possible that in an *in vivo* situation, the differences in

TABLE II. Effect of Mycoplasma on Virus Plaque Formation.

Challenge virus	MEF			L cells		
		Infected with			Infected with	
	Mycoplasma free	*M. hyorhinis*	*M. arginini*	Mycoplasma free	*M. hyorhinis*	*M. arginini*
VSV	57[a]	16	28	87	89	95
	56	27	29	125	156	144
SFV	103	17	35	46	53	49
	64	11	18	129	105	120

[a] Average plaque count from three or more bottles per group. Data represent two separate experiments per box.

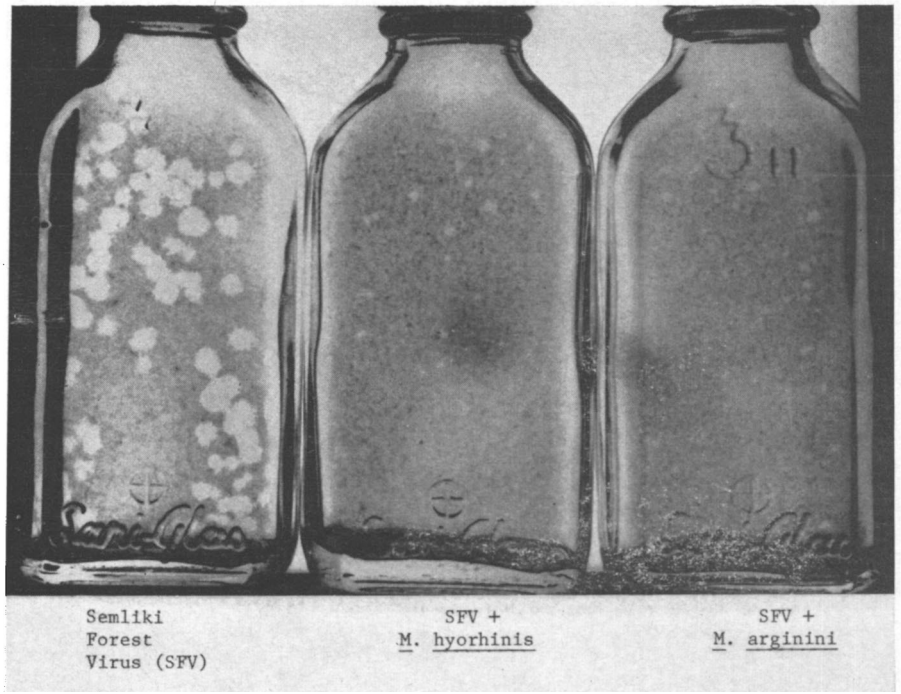

FIG. 1. Effect of mycoplasma on plaque morphology and number in MEF cells.

virus replication in mycoplasma carrying hosts could markedly influence the outcome of the virus disease.

These data indicate, as do increasing reports in the literature, that mycoplasma contamination of cell cultures is not always innocuous. Although mycoplasma contamination may have no *observable* effect on virus replication or plaque formation, it is becoming clear that such contamination indeed can effect increased or decreased virus yields and alterations in plaque morphology.

In addition, the cell type on which the virus–mycoplasma interaction takes place would appear to be an important factor in determining the outcome of the interaction.

Summary. Significantly increased as well as decreased virus yields were obtained from both mouse embryo fibroblasts and L cells chronically infected with mycoplasma. The determination of the direction of change was influenced not only by the particular mycoplasma and virus used in the experiments, but also by the cell type in which the interaction took place. Plaque morphology and count were also capable of being influenced by chronic mycoplasma infection.

1. Singer, S. H., Kirschstein, R. L., and Barile, M. F., Nature (London) **222**, 1087 (1969).

2. Singer, S. H., Barile, M. F., and Kirschstein, R. L., Proc. Soc. Exp. Biol. Med. **131**, 1129 (1969).

3. Hargreaves, F. D., and Leach, R. H., J. Med. Microbiol. **3**, 259 (1970).

4. Rouse, H. C., Valentin, B. H., and Schlesinger, W. R., Virology **20**, 357 (1963).

5. Butler, M., and Leach, R. H., J. Gen. Microbiol. **34**, 285 (1964).

6. Somerson, N. L., and Cook, M. K., J. Bacteriol. **90**, 534 (1965).

7. Kagen, G. Y., Ann. N.Y. Acad. Sci. **143**, 734 (1967).

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