Acholeplasma laidlawii Cells Acutely and Chronically Infected with Group 1 Acholeplasmavirus

By ALAN LISS* AND BRENDA E. RITTER

Department of Biological Science, University Center at Binghamton, State University of New York, Binghamton, NY 13901, USA

(Received 26 September 1984; revised 12 February 1985)

Interactions between group 1 acholeplasmaviruses and their host cells were studied. Acutely infected, chronically infected and uninfected cultures of Acholeplasma laidlawii strain JA1 were compared by their growth in broth and on agar, by the sensitivities of the uninfected and chronically infected cells to representatives of each of the three groups of acholeplasmaviruses, and by their SDS-PAGE polypeptide profiles. Acutely infected cells resembled uninfected cells by these criteria, except for the fact that progeny virus was being released. Two types of chronically infected cells were found: rapid growers (the same doubling time as uninfected cells) and slow growers. The latter resembled uninfected cells, except for their slower growth and low-level release of virus, and the former was resistant to group 1 viruses and had a unique polypeptide profile. These biological characterizations help to establish the non-lytic, non-cytocidal cycle of the group 1 acholeplasmaviruses.

INTRODUCTION

Acholeplasma laidlawii (class Mollicutes) can be infected by one or more acholeplasmaviruses. These virus isolates can be placed into three distinct groups: group 1 is a naked, bullet-shaped virus; group 2 is enveloped without a distinct nucleocapsid; and group 3 is a complex virus having an icosahedral head and a short tail. These viruses have been placed in the Inoviridae, Plasmaviridae and Pedoviridae taxonomic families, respectively (Matthews, 1979). Typical bacteriophage life cycles involve intracellular assembly with progeny phage release as a consequence of host cell lysis. Intracellular virus assembly appears only with group 3 acholeplasmaviruses. Assembly of group 1 particles appears similar to that in filamentous eubacterial viruses. Assembly of group 2 particles resembles that in budding animal viruses. Virus release through lysis of infected cells does not occur in these acholeplasmaviruses (Maniloff et al., 1982). At the intracellular level, group 2 viruses may integrate their genome into that of their host (i.e. lysogeny; Dybvig & Maniloff, 1983). There is no evidence for a similar intracellular relationship between virus and host for group 1 or group 3 acholeplasmaviruses.

The group 1 acholeplasmavirus-A. laidlawii host system has been the most thoroughly investigated of the three virus groups. Virus-infected cells appear to double at a rate slower than that of uninfected cells (Liss & Maniloff, 1973). Infected cells can form colonies which are obviously different from uninfected cells (Congdon et al., 1979; Maniloff & Liss, 1973; Milne et al., 1972). The non-lytic, non-cytocidal nature of these viruses creates a situation where the host can be chronically infected (Liss & Maniloff, 1973; Roger, 1977, 1982, 1983).

We have investigated the interaction of the group 1 virus MVL51 with the host A. laidlawii strain JA1. Three general conditions were studied: (1) host cells which have a prolonged, chronic virus infection; (2) host cells which are actively releasing progeny virus (defined as an acute infection); and (3) the control, uninfected cells. Cells in each of the above categories were compared by their growth in broth and on agar, by their sensitivities to reference virus and by their sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) polypeptide profiles.
Table 1. *Acholeplasma laidlawii* strains and their sensitivity to *achoyleplasmavirus*

Each test was done five times with identical results; +, a spot of reference virus cleared the test lawn; ±, a spot of reference virus produced plaques but not confluent clearing; −, a spot of reference virus did not clear the test lawn.

<table>
<thead>
<tr>
<th>Strain designation</th>
<th>Sensitivity to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MVL51</td>
</tr>
<tr>
<td>JA1</td>
<td>+</td>
</tr>
<tr>
<td>JSY</td>
<td>−</td>
</tr>
<tr>
<td>JA1(1R)</td>
<td>+</td>
</tr>
<tr>
<td>Jgct</td>
<td>−</td>
</tr>
<tr>
<td>Jcct</td>
<td>+</td>
</tr>
</tbody>
</table>

**METHODS**

*Cells and viruses.* The *Acholeplasma laidlawii* strains used are listed in Table 1. All were derived from strain JA1 cultures and confirmed to be *A. laidlawii* by growth inhibition using antibody against strain B (ju) and by the absence of a sterol requirement (WHO Working Group, 1976). Cloned strains of virus from achoyleplasmavirus groups 1, 2 and 3, designated MVL51 (Liss & Maniloff, 1973), MVL2 (Gourlay, 1971) and MVL3 (Liss, 1977), respectively, were also used. Virus and cells were assayed as plaque forming units (p.f.u.) or colony forming units (c.f.u.) as previously described (Liss & Maniloff, 1971).

Acutely infected cells were generated by infecting strain JA1 with MVL51 at a multiplicity of infection (m.o.i.) of about 5 (Steinick et al., 1980). Most tests were done within 23 to 30 h post infection with no additional virus being added to these cell cultures. Chronically infected cells [represented by strains JSY and JA1(1R)] were generated by infecting strain JA1 with MVL51 at an m.o.i. of 5 to 10. These cultures were serially passaged into fresh medium which contained additional MVL51 (10⁵ p.f.u. ml⁻¹) every 20 h for 7 d. These cells were then plated on solid medium. Resulting colonies were then selected, propagated in broth (free of virus) and used for further study. The uninfected JA1 broth culture was generated from a single colony and serially passed about 100 times in broth medium. After plating a portion of a broth culture on tryptose agar (cf. below), a variety of colony morphologies was observed. We did not reclone strain JA1 for these studies. Instead, a colony representative of the numerically dominant colonial phenotype (a 'typical' fried egg shaped colony with a clear, even periphery; Fig. 1a) was selected and propagated in broth. The resulting broth culture was called Jcct (clear colony type). The colonial phenotype was stable for up to 15 daily broth passages. A colony representative of a minority colonial phenotype (a fried egg shaped colony with a granular appearance; Fig. 1b) was also selected. This phenotype was stable for up to 15 daily passages and was designated strain Jgct (granular colony type). Morphologically similar colonies (10 of each phenotype) were tested for virus sensitivity and SDS–PAGE polypeptide profile. Each culture appeared to be similar to the appropriate prototype strains (data not shown).

*Growth conditions.* All strains were grown in tryptose medium supplemented with 1% (v/v) PPLO serum fraction (Difco) as noted by Maniloff (1969). Incubation of broth or agar [when the above medium was supplemented with 1% (w/v) Bacto-agar (Difco)] cultures was at 37 °C in air.

*Biological studies.* Cell growth was determined as c.f.u. versus time (Maniloff, 1969). Virus adsorption to each cell type was determined (Liss & Heiland, 1982) using a constant virus concentration of 10⁶ p.f.u. ml⁻¹ and a cell concentration of 10⁸ c.f.u. ml⁻¹. Unadsorbed virus was determined by titrating culture supernatants after centrifugation at 4 °C for 10 min in an Eppendorf microtube (model 5414; Brinkmann Instruments, Westbury, NY, USA). Mitomycin C was used to attempt virus induction from host cells (Liss, 1981). Each strain was tested for sensitivity to reference achoyleplasmavirus stocks. Approximately 10⁵ p.f.u. ml⁻¹ of MVL51, MVL2 and MVL3 were each applied to dried lawns and the agar plates were incubated at 37 °C. After 24 h, sensitivity to virus was demonstrated by a zone of clearing on the test lawn. Each test was done five times.

*SDS–PAGE.* Broth cultures of each cell type were concentrated by centrifugation. Polypeptide profiles of these cell preparations were obtained by SDS–PAGE (Liss & Heiland, 1982). Molecular weight standards (low molecular weight set; Bio-Rad) were run along with test samples. Polypeptides were stained with Coomassie Brilliant Blue G (Serva Biochemicals, Garden City Park, NJ, USA).

**RESULTS**

*Growth on solid medium.* All the strains used were capable of forming colonies on tryptose agar after 3 to 5 d incubation at 37 °C. As noted above, JA1 cultures contained a variety of colonial morphologies. Agar passage of colonies resembling Jcct (Fig. 1a) or Jgct (Fig. 1b) gave
Group 1 acholeplasmavirus-host interactions

reproducible colonial phenotypes for at least 50 consecutive agar passages. Using chronically infected cultures, we found that colonial morphology was also a stable phenotypic character. Selecting 10 large colonies (1 mm or more after 7 d incubation) from a chronically infected JA1 culture resulted in cultures typified by JSY. Selecting 10 small colonies (0.5 mm or less after 10 d incubation) resulted in cultures typified by JA1(1R). JSY cultures had colonial morphologies identical to those of Jgct, and JA1(1R) colonies were smaller than, but otherwise resembled, Jcct colonies. When cultivated in broth for 20 or more passages each strain noted above produced a culture which resembled the JA1 culture in regard to the variety of colonial morphologies generated.

Growth in broth medium. All the cultures tested could be serially passaged and maintained in broth culture. Uninfected cultures of JA1, Jcct and Jgct reached a maximum cell titre of about $1 \times 10^9$ c.f.u. ml$^{-1}$ 20 h after transfer to fresh medium. The chronically infected cultures JA1(1R) and JSY had a maximum titre of $5 \times 10^8$ c.f.u. ml$^{-1}$ at 30 h and 20 h, respectively. Acutely infected cells reached a titre of $1 \times 10^8$ c.f.u. ml$^{-1}$ at 20 h ($n = 7$). Doubling times of exponential phase cultures incubated in broth at 37 °C were also determined. Cultures of JA1, JSY, Jgct and Jcct doubled approximately every 95 min. Slower growth was observed for the chronically infected JA1(1R) which doubled approximately every 215 min.

Virus sensitivities of hosts. All host strains were sensitive to the group 2 virus MVL2 (Table 1). Strains JA1, Jcct and JA1(1R) were also sensitive to group 1 and group 3 viruses, MVL51 and MVL3 respectively. Strains JSY and Jgct, however, were resistant to MVL51 and less sensitive to MVL3 than the other host strains. Acutely infected cells were not tested.
Both JSY and Jgct were tested for the ability to adsorb MVL51, to see if the initial virus-host contact stage might be aberrant, leading to the inability of these hosts to propagate group 1 viruses. Free (unadsorbed) virus was less than 1% of the original titre after 5 min at 37°C for all cells tested. At 10 min no free virus could be detected.

Virus induction from host cell cultures. A JA1 derivative (JA2) has been shown previously to carry a mitomycin C inducible group 1 virus (Liss, 1981). A group 1-type virus can also be induced from JA1 and Jcct with mitomycin C. Free virus was not present in JA1 and Jcct cultures that were not treated with mitomycin C. Although free virus was found in acutely infected cells (as noted in the definition) as well as in acutely infected cultures of JA1(1R), no free or induced virus was found in JSY or Jgct cultures. Virus releasing strains do make confluent lawns free of spontaneous plaques. Washing the lawns with tryptose broth medium showed that virus was being released from these cells on agar as well as in broth culture. Using differentially sensitive hosts (Liss, 1981), the virus being released from all the strains noted above was of the group 1 type.

SDS-PAGE analysis. Polypeptide profiles were nearly identical for all the strains examined (Fig. 2). However, in the appropriate molecular weight range of 35 to 40 kDa there were differences. Tracks A (JA1 acutely infected with MVL51), B [JA1(1R)], D (Jcct) and F (uninfected JA1) had two dominant bands in this region of the gel. Tracks C (JSY) and E (Jgct) had a broad band approximately bisecting the two bands noted above (Fig. 2).
DISCUSSION

In the eubacteria, a clear cut situation usually exists between an uninfected and an infected host cell. Generally this is because many bacteriophages are released from their host by lysis of the infected cell, while in some bacteriophage systems a non-productive state called lysogeny can exist. Exceptions to this lytic-lysogenic organization are found in the filamentous bacteriophages (Denhardt, 1978). Similar growth characteristics between group 1 acholeplasmaviruses and filamentous viruses have been noted (Maniloff et al., 1978, 1979).

The cell culture characteristics reported here are consistent with the observation that group 1 acholeplasmaviruses resemble filamentous Inoviridae, in that productive infection does not lead to host cell death. Chronically infected cells can be generated without equating this situation to the non-productive stage of temperature eubacterial bacteriophage defined under the term lysogeny. Interestingly, what we call uninfected cells are really *A. laidlawii* strain JAl carrying group 1 viral genomes in a stable but inducible form (Liss, 1981). However, JAl cultures are significantly different from our chronically infected cells. We are currently attempting to 're-isolate' a JAl-type cell from JSY and Jgct cultures. Successful isolation would suggest that a seemingly uninfected cell is the natural progression from an unstable chronic infection to a stable, non-virus releasing, chronically infected cell type.

Colonial phenotypes, indicative of cell-to-cell association, have been valuable in identifying a variety of cell mutants in eubacteria and mycoplasmas (e.g. Liss & Heiland, 1983). Colonial morphologies were found to be affected by the viral phase. Comparisons of Jgct and Jcct cultures gave the most obvious differences in colonial morphology in this regard. Although many factors must be involved, Jgct cultures form colonies made of fewer cells than do Jcct cultures (Clark et al., 1985). We are currently investigating the biochemical basis for the colonial morphology variation that we and others (Congdon et al., 1979; Milne et al., 1972) have reported.

Chronically infected JSY cells were resistant to homologous virus but JAl(1R) cells were not. Also, JAl(1R) produced plaque-free lawns on agar medium even though these cells released group 1 virus into culture supernatants. Filtrates of JAl(1R) will form plaques on JAl(1R) lawns yet spontaneous plaques on test lawns are infrequently observed (Liss et al., 1985).

In contrast, JAl cells lysogenically carrying a group 2 acholeplasmavirus, MVL2, apparently are always immune to homologous virus (Putzrath & Maniloff, 1977). In this study, homogeneity of the group 2-resistant cultures was not established, making a comparison with our results difficult.

Virus induction with mitomycin C was observed for *A. laidlawii* strains JAl and Jcct but not for strains JSY or Jgct. Jcct-like cells do constitute the major colonial morphology found in JAl cultures, and may be similar in many other ways. JSY and Jgct are experimentally derived from JAl cultures, but may have lost an inducible virus found in the parent JAl culture. Alternatively, these cultures may resemble the non-inducible filamentous phage mutants of *Escherichia coli* that were reported by Hsu (1968). More studies on this intracellular virus relationship are necessary.

SDS–PAGE profiles separated the strains noted here into two groups: those resembling JAl [strains JAl, JAl(1R) and Jcct] and those resembling Jgct (JSY and Jgct). Neither chronically infected cell population showed polypeptide bands corresponding to the MVL51 virion components that were reported previously (Maniloff et al., 1978).

In conclusion, group 1 acholeplasmavirus infection of *A. laidlawii* JAl can be described in terms of an acute and a chronic phase. The virus–host relationships of this system resemble, but are not identical with, the filamentous phage of eubacteria. The group 1 acholeplasmavirus model system differs from the eubacterial one especially in regard to the lack of a cell wall or outer membrane of the host cell. Problems involving acute virus release or chronic virus–host cell relationships in a cell bounded by a single membrane (as in animal cells) may be studied using the virus and host model presented here. Clearly, more investigations are needed to explore fully the non-lytic, non-cytocidal viruses of the cell wall-less prokaryotes.
Part of this research was funded by a BRSG grant no. S07RR07149-10 awarded by the Biomedical Research Support Grant Program and a grant from the State University of New York Research Foundation University Awards Program. We thank Valeria Junqueira for her fine technical assistance and Anna Sevcovic and Mary Kurey for assistance in the preparation of this manuscript.

REFERENCES


