

Spontaneous Change from Overt to Covert Infection of *Chlamydia pecorum* in Cycloheximide-Treated Mouse McCoy Cells

H. L. PHILIPS AND M. J. CLARKSON*

Department of Veterinary Clinical Science and Animal Husbandry, University of Liverpool, Leahurst, Neston, South Wirral, L64 7TE, England

Received 14 March 1995/Returned for modification 16 May 1995/Accepted 22 June 1995

Some isolates of *Chlamydia pecorum* from sheep feces failed to produce inclusions on passage in cycloheximide-treated monolayers, but chlamydiae could be recovered several weeks later. *Chlamydia psittaci* from sheep abortions did not show this phenomenon.

Diseases caused by chlamydiae, such as trachoma, sexually transmitted diseases, and psittacosis, are characterized by a chronic, latent, or relapsing course (12). Chlamydiae may be excreted by an apparently healthy host and may also persist in a cryptic form (9). The phenomenon of persistence in a cryptic form has been investigated by in vitro techniques. Persistently infected populations of mouse fibroblasts (L cells) were established with the avian *Chlamydia psittaci* strain 6BC and consisted mainly of inclusion-free L cells which grew more slowly than uninfected cells (4). They were almost completely resistant to superinfection with the same strain. Resistance to superinfection was found to be associated with changes in cell surface structure (5).

In cell culture, one form of the latent state is revealed by the effect of cycloheximide, which enhances the proportion of productively infected cells. As increasing the number of chlamydiae inoculated in the absence of cycloheximide will usually lead to infection of all the host cells, the fact that cycloheximide increases the proportion of inclusion-bearing cells cannot be due to differences in host cell susceptibility but it suggests that each organism-host cell interaction leads to internalized chlamydiae with various capacities to multiply (6).

Isolates of both abortion and enteric chlamydiae from ruminants are able to persistently infect McCoy cells in the absence of cycloheximide (8).

In this study, several isolates of *Chlamydia pecorum* were observed, after a number of passages, to manifest themselves spontaneously as a covert infection which spontaneously reverted back to overt infection.

Sources of chlamydiae. (i) *C. pecorum*. Feces samples were taken from the rectums of 20 lambs, aged 6 to 7 months, from each of 10 farms in Wales by using a separate disposable glove for each lamb. Approximately 0.2 g of feces was suspended in 2 ml of transport medium (11).

(ii) *C. psittaci*. Fetal membranes were sampled from outbreaks of abortion on 35 farms in England and Wales. Pieces of tissues approximately 0.5 cm in diameter were placed in 2 ml of transport medium.

Growing chlamydiae. The chlamydiae were grown according to a previously reported method (2). If inclusions were not detected after passage from a culture known to be positive, a duplicate culture was trypsinized and the cells were resuspended in 199 medium with 10% fetal calf serum and without

cycloheximide. At weekly intervals, 100 μ l of supernatant medium was inoculated onto fresh monolayers.

Pure cultures. Pure cultures of chlamydiae were obtained from three isolates of *C. pecorum* and one isolate of *C. psittaci*. McCoy cells that had previously been infected with chlamydiae sufficient to infect about 75% of the cells and incubated for 18 h were trypsinized and resuspended in phosphate-buffered saline containing 10% fetal calf serum. A sterile glass Pasteur pipette which had been drawn out over a Bunsen burner was used to pick up individual McCoy cells and resuspend them in a small volume of 199 medium containing 10% fetal calf serum and 1 μ g of cycloheximide per ml. The cells were incubated for 3 to 4 days and then passaged in the usual way.

Thirty-one of the 200 feces samples from the lambs were positive for chlamydiae. When these were passaged, 4 of the isolates failed to grow, usually after two or three passages, whereas the other 27 could be subpassaged successfully. Twenty-three pure cultures were obtained from isolates of *C. pecorum*. Four of these became covert in that the whole population disappeared after six to eight passages. After the duplicate culture was trypsinized and allowed to grow, chlamydiae could be isolated from the supernatant medium after a few weeks, although there was no cytopathic effect unless cycloheximide was added. Chlamydiae isolated from the culture did not become covert in three to four subsequent passages. In contrast, all 35 abortion isolates could be passaged successfully. No covert infections occurred in 11 cultures derived from single cells infected with an abortion isolate of *C. psittaci*.

Persistent infections in the absence of inhibitors or deficiencies have been established in a number of ways, and no single mechanism can account for them all (3). For example, the latent state can be brought about by an insufficiency of isoleucine, and the addition of isoleucine or cycloheximide activated the latent chlamydial infection (1); persistent infections of L cells by an ovine abortion strain of *C. psittaci* could be activated by the addition of cycloheximide (7); and the latency of strain 6BC in mouse macrophages could be reversed by increasing the temperature (10).

The spontaneous disappearance of *C. pecorum* described here differs from the results of previous studies in that the cause could not have been a nutritional deficiency or another variant in culture conditions, since the chlamydiae became covert and later grew actively under uniform conditions. In addition, the phenomenon occurred in the presence of cycloheximide, which generally enhances the proportion of productively infected cells (6).

* Corresponding author. Phone: 0151 794 6023. Fax: 0151 794 6029.

It is not obvious what causes this change from overt to covert infection and its reversal, but it is possible that the cause took place several generations before the result became apparent, since whole populations showed the change at the same time.

We are grateful to the Agricultural and Food Research Council for the provision of a grant.

REFERENCES

1. **Hatch, T. P.** 1975. Competition between *Chlamydia psittaci* and L cells for host isoleucine pools: a limiting factor in chlamydial multiplication. *Infect. Immun.* **12**:211–220.
2. **Johnson, F. W. A., M. J. Clarkson, and W. N. Spencer.** 1983. Direct isolation of the agent of enzootic abortion of ewes (*Chlamydia psittaci*) in cell culture. *Vet. Rec.* **113**:413–414.
3. **Moulder, J. W.** 1991. Interaction of chlamydiae and host cells in vitro. *Microbiol. Rev.* **55**:143–190.
4. **Moulder, J. W., N. J. Levy, and L. P. Schulman.** 1980. Persistent infection of mouse fibroblasts (L cells) with *Chlamydia psittaci*: evidence for a cryptic chlamydial form. *Infect. Immun.* **30**:874–883.
5. **Moulder, J. W., S. L. Zeichner, and N. J. Levy.** 1982. Association between resistance to superinfection and patterns of surface protein labeling in mouse fibroblasts (L cells) persistently infected with *Chlamydia psittaci*. *Infect. Immun.* **35**:834–839.
6. **Pearce, J. H.** 1988. Chlamydial invasion mechanisms: implications for parasite growth and host restraint. *Proc. Eur. Soc. Chlamydia Res.* **1**:109–112.
7. **Perez-Martinez, J. A., and J. Storz.** 1985. Persistent infection of L cells with an ovine abortion strain of *Chlamydia psittaci*. *Infect. Immun.* **50**:453–458.
8. **Rodolakis, A., F. Bernard, A. Souriau, K. Layachi, and D. Buzoni-Gatel.** 1989. Relationship between virulence of *Chlamydia psittaci* strains and establishment of persistent infection of McCoy cells. *Vet. Microbiol.* **19**:65–73.
9. **Schachter, J., J. Storz, M. L. Tarizzo, and K. Bogel.** 1973. Chlamydia as agents of human and animal disease. *Bull. W.H.O.* **49**:443–449.
10. **Schoenholtz, W. K.** 1969. Studies on *Bedsonia* latency. II. Effect of immune lymphocytes and of rabbit-anti-lymphocyte globulin (RAMLG) on infected macrophages exposed to increased incubation temperature in vitro. *Z. Immunitaetsforsch.* **139**:359–371.
11. **Spencer, W. N., and F. W. A. Johnson.** 1983. A simple transport medium for the isolation of chlamydia from clinical material. *Vet. Rec.* **113**:535–536.
12. **Storz, J.** 1971. Chlamydia and chlamydia induced diseases. Charles C. Thomas, Springfield, Ill.