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ANALYSIS OF *COXIELLA BURNETII* ISOLATES IN CELL CULTURE AND THE EXPRESSION OF PARASITE-SPECIFIC ANTIGENS ON THE HOST MEMBRANE SURFACE

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Summary. - *Coxiella burnetii* isolates may be classified into several groups based on plasmid character. These groups may also be correlated with disease syndrome - chronic or short-term acute. L929 mouse fibroblast cells were exposed, independently, to two members of the three major *C. burnetii* groups, and their growth /morphological characteristics analysed by light and electron microscopy, including High Voltage Electron Microscopy. The fates of the isolates were followed. Two acute isolates [Nine Mile (RSA 493) and Henzerling (RSA 331), QpH1-type plasmids] and two chronic isolates (S Q217 and L Q216, plasmidless) readily infected L929 cells in static culture. Priscilla (Q177) and F (Q228) isolates (QpRS-type plasmid, and implicated in causing chronic Q fever) took longer to infect cells, and, unlike the members of the other two groups, gradually disappeared when shifted to suspension culture. Cells infected with Q177 and Q228 exhibited a higher degree of vacuolation than cells infected with the other isolates. Parasite-specific antigens on the surfaces of the host cells were analysed by immunofluorescence/flow cytometry. The acute and plasmidless isolates caused the display of significantly more *C. burnetii*-specific antigen on the host cell membrane than the two QpRS plasmid-containing isolates. This host cell model system clearly reveals biological differences among the *C. burnetii* groups.

Key words: C. burnetii isolates; classification; plasmids; surface antigens; flow cytometry

Introduction

The etiological agent of Q fever, *Coxiella burnetii*, is an obligate intracellular parasite (Derrick, 1937). Unlike other members of the family Rickettsiaceae,

C. burnetii resides within the phagolysosome of host cells (Burton *et al.*, 1971; Akporiaye *et al.*, 1983). All *C. burnetii* isolates studied thus far can be classified into three major groups based on the type or lack of plasmid (Samuel *et al.*, 1985) and, also, on their lipopolysaccharide profiles (Moos and Hackstadt, 1987). Furthermore, these three groups are associated with different disease syndromes, including acute and chronic disease. For example, the Nine Mile (RSA 493) and Henzerling (RSA 331) isolates have the QpH1-type plasmid and are implicated in causing acute disease. The Priscilla (Q177) and F (Q228) isolates belong to another group that contains a larger plasmid (designated QpRS) and, along with the third plasmidless isolates, S (Q217) and L (Q216), are associated with chronic disease.

No adequate *in vivo* model is available to study Q fever. In our laboratory, we have developed an *in vitro* model consting of host cells infected with *C. burnetii* (Baca *et al.*, 1981; Roman *et al.*, 1986). Using this model we investigated the growth/morphological characteristics of host cells infected with six different *C. burnetii* isolates (two isolates from each of the three major groups). Previously, it was suggested that rickettsial antigens may be inserted into host cell membranes (Marecky *et al.*, 1978; Koster *et al.*, 1985). This was based on the detection of different electrophoretic profiles of membrane proteins from normal and infected L929 and liver cells and antibody dependent cell cytotoxicity (ADCC). In this report, we also present evidence that, indeed, *C. burnetii*-specific antigens are expressed on the surface of infected cells.

Materials and Methods

L929 mouse fibroblast cells were infected separately with six *C. burnetii* isolates [Nine Mile (RSA 493), Henzerling (RSA 331), Priscilla (Q177), F (Q228), S (Q217), and L (Q216)]. After initial infection with *C. burnetii*, the cells were passaged three times a week and followed for over 100 days. To determine per cent of cells infected, samples were centrifuged onto slides with a Cytospin 2 and stained by the Gimenez method (Gimenez, 1964). Cells were prepared also for electron microscopy using standard methods.

Specific antibody vs. *C. burnetii* coupled with fluorescence microscopy and flow cytometry was used in this investigation to determine if rickettsial antigens are inserted into the host cell membrane. Cells were treated with serum (normal or anti-*C. burnetii* human serum) for 15 min. The cells were subsequently washed with PBS-BSA-azide solution (phosphate buffer saline with 0.5 % bovine serum albumin and 0.1 % sodium azide), treated with tagged anti-IgG (Texas Red) for 15 min, fixed for 1 hr with paraformaldehyde, and finally resuspended in PBS. These cells were then examined with a three-laser flow cytometric system at Los Alamos National Laboratories, Los Alamos, NM. Ten thousand cells were examined from each cell population and the fluorescence intensity was measured.

Results and Discussion

All six *Coxiella burnetii* isolates were able to infect L929 mouse fibroblast cells. The QpH1 group (Nine Mile and Henzerling) and the plasmidless group

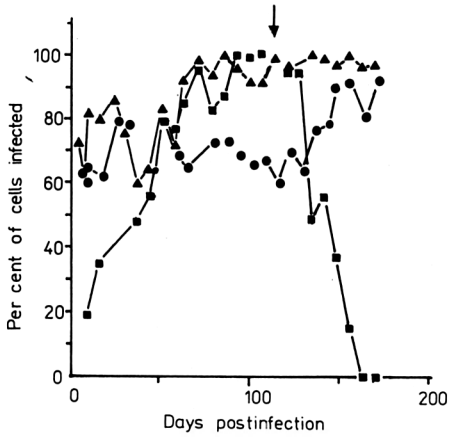


Fig. 1
Comparison of the per cent infection of L929 cells with the Nine Mile (●), Priscilla (■), and S (▲) isolates of *C. burnetii* during the first 170 days post-infection
The per cent of L929 cells infected with the Nine Mile isolate was approximately 60 % after the first four days and subsequently fluctuated between 60 and 100 %. Priscilla-infected cells took longer to infect and, when transferred from static to suspension culture (arrowed), lost the parasite. Cells infected with the S isolate at day 4 were 70 % infected and then varied between 60 and 100 %.

(S and L) were able to readily infect the cells in static culture, while the QpRS group (Priscilla and F) took longer to infect. The per cent of total cells infected (with the six isolates) over time are shown in Figs. 1 and 2. At day 2, Nine Mile-, Henzerling-, S-, and L-infected cells were over 40 % infected, while the Priscilla and F organisms could not be detected until day 10. Ten per cent of these cell populations had at least 50 rickettsiae per cell. Even at day 10, they had a low degree of infectivity (only about 20 %). When Priscilla- and F-infected cells were transferred from static to suspension culture, the parasites disappeared gradually by approximately 2 months. This was not the case with the other two groups which can be maintained in suspension culture (Figs. 1 and 2).

The host cell population did not seem affected even with the large parasitic load, since they were able to undergo mitosis followed by cytokinesis. Depending on the infecting isolate, the number of rickettsiae-containing vacuoles

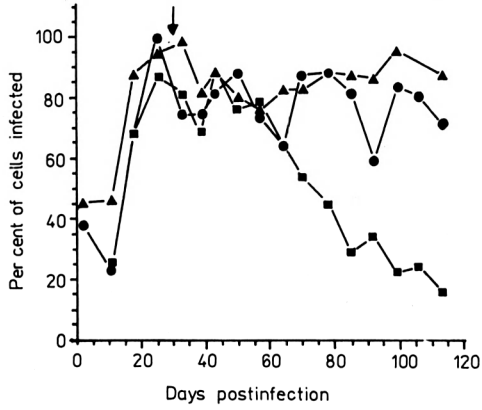
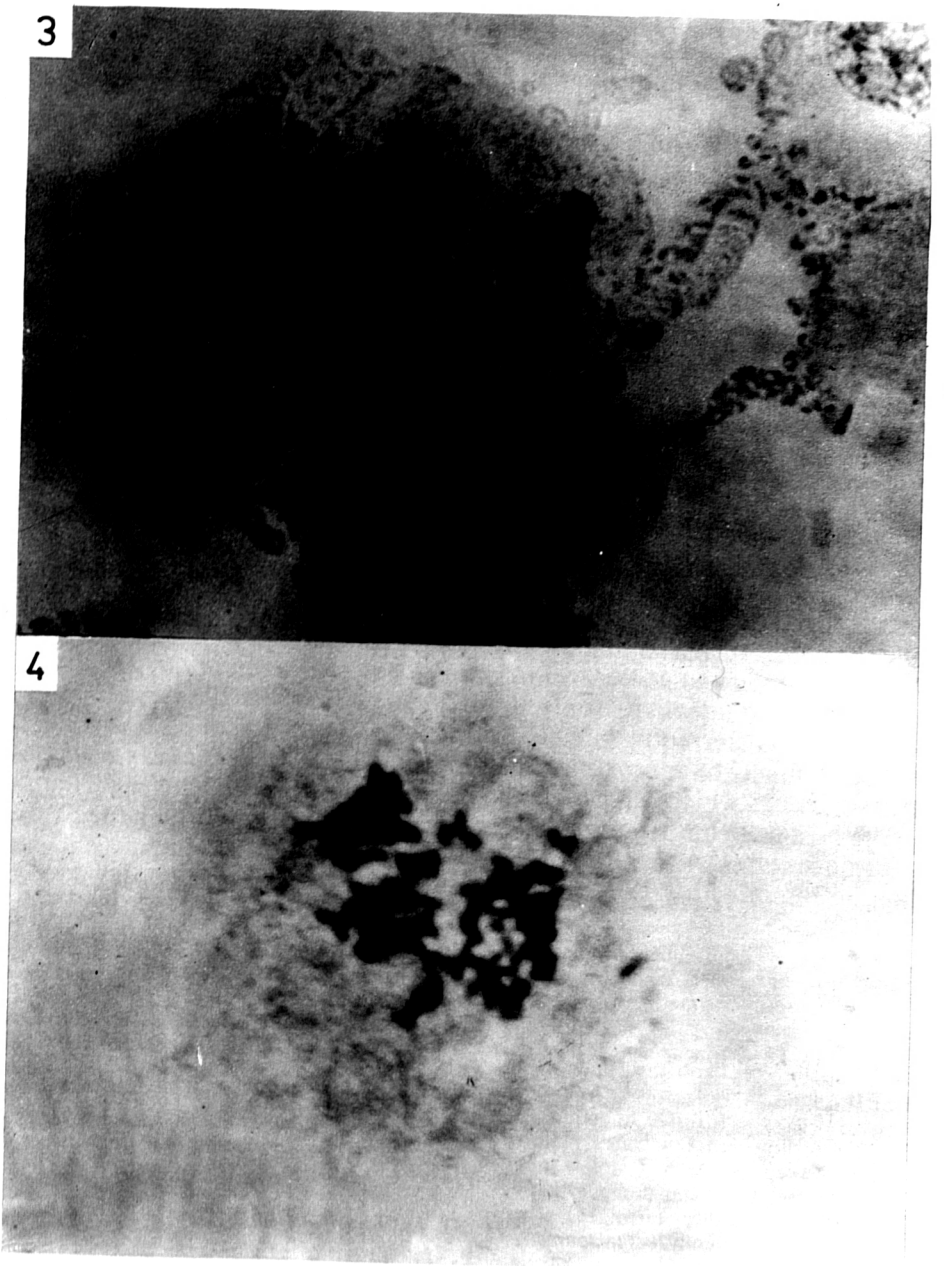


Fig. 2
L929 cells infected with the Henzerling (●), F (■), and L (▲) isolates were followed for 115 days after initial infection
The Henzerling and L isolates readily infected the cells, while the F isolate took longer to infect. Upon transfer from static to suspension culture (arrowed), the F-infected cells gradually lost the parasite while the Henzerling and L-infected cells remained infected. The per cent of cells infected fluctuated between 40 and 100 %.



Figs. 3–4
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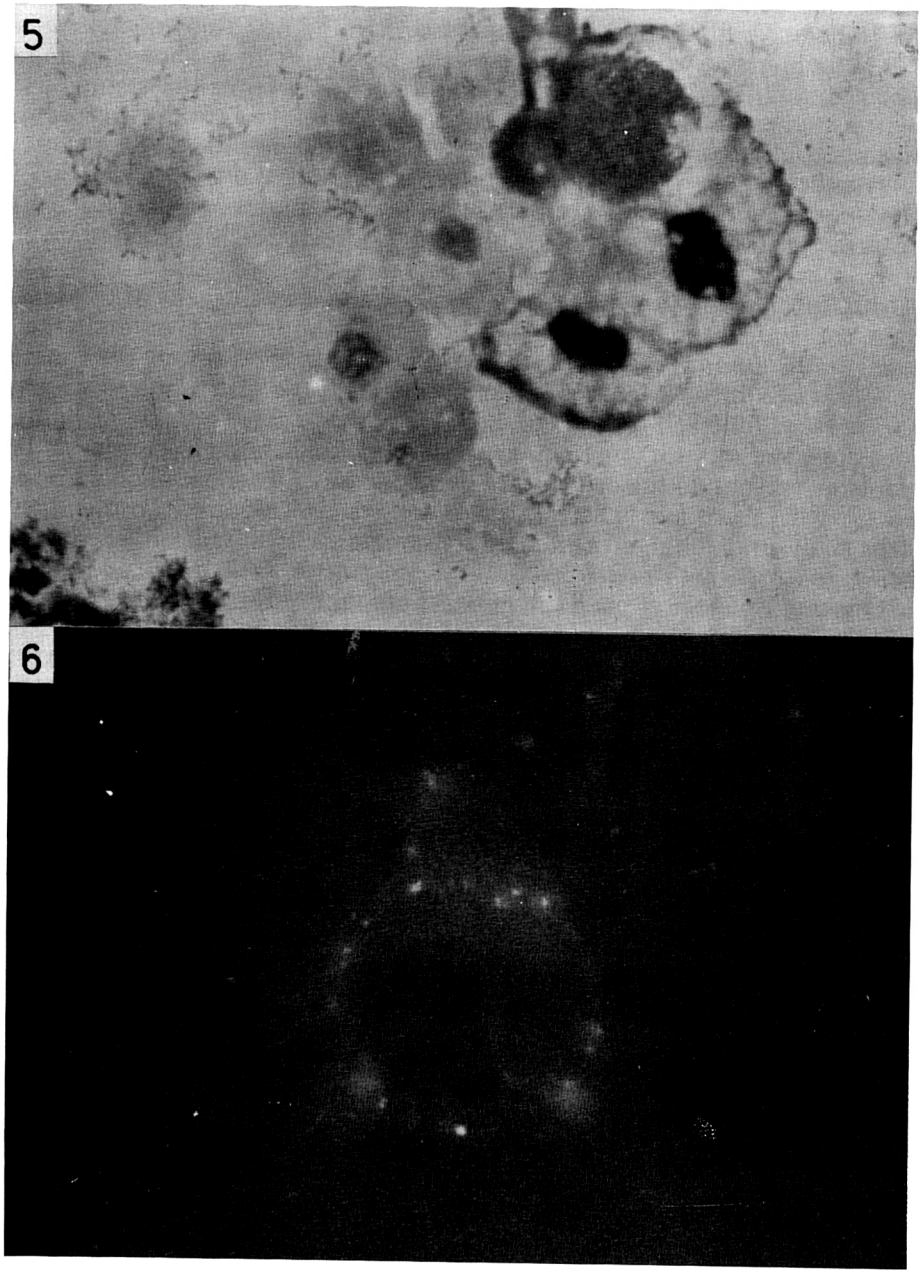

Figs. 5–6
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varied. Members of the acute and chronic (plasmidless) groups of *C. burnetii* usually had one highly infected vacuole containing hundreds of rickettsiae. L929 cells infected with a member of the QpH1-group is shown in Fig. 3 (Henzerling at day 43 post-infection). Fig. 4 illustrates Gimenez-stained L929 cells infected with the L isolate (member of the plasmidless group). A representative photomicrograph of cells infected with a member of the QpRS group (F) shows infected cells with several vacuoles containing the rickettsiae (Fig. 5). Cells infected with members of the QpH1 and plasmidless group usually had one large highly infected vacuole, while QpRS-infected cells contained several highly infected vacuoles.

Using specific antibody to *C. burnetii*, we found that antigens of *C. burnetii* origin are, indeed, inserted into the host cell membrane of infected cells. However, the amount of surface antigen expressed varied among the isolates. Fig. 6 shows S-infected L929 cells (for 365 days) treated with human antiserum to *C. burnetii* followed by FITC-labelled conjugate. The peripheral fluorescence observed was not due to intact rickettsiae; concerted efforts revealed no organisms present on the cell surface (similar results were obtained with cells infected with the other isolates). Fig. 7 depicts a histogram from flow cytometric analyses of long-term infected cells treated with human antiserum to *C. burnetii* followed by Texas Red-anti-IgG conjugate. Cells infected with the QpH1 group isolates revealed the greatest amount of fluorescence, while the QpRS group isolates exhibited the least amount of fluorescence; the plasmidless group isolates had intermediate levels of fluorescence. This suggests that, depending on the infecting isolate and its group association, different amounts of antigens are inserted into the host cell membrane. There was no significant difference in fluorescence intensity between long- and short-term infected cells (data not shown).

While the QpH1 (Nine Mile and Henzerling) and plasmidless (S and L) isolates can readily infect L929 fibroblast cells, the QpRS isolates (Priscilla and F) take longer to establish infection. Also, unlike the other isolates, the QpRS isolates of *C. burnetii* gradually disappear from culture when transferred from static to suspension culture. It was also found that Priscilla- and F-infected

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Fig. 3. Photomicrograph of L929 cells infected with the Henzerling isolate for 43 days. Cell in telophase has several small rickettsiae-containing vacuoles. Magn. × 5 500.

Fig. 4. Gimenez stain of F-L929 infected cells from a population that was infected for 213 days. Several vacuoles are found in this F-infected cell in an early stage of mitosis. Magn. × 5 500.

Fig. 5. Photomicrograph of L929 cells infected with the L isolates that had been infected for 213 days. Cell in late telophase is giving rise to an infected cell and a normal uninfected cell. Magn. × 5 500.

Fig. 6. L929 mouse fibroblast cells infected with the S isolate of *C. burnetii* for 365 days. The cells were treated with S-antiserum followed by anti-human-IgG-FITC. Note peripheral fluorescence. Magn. × 6 250.

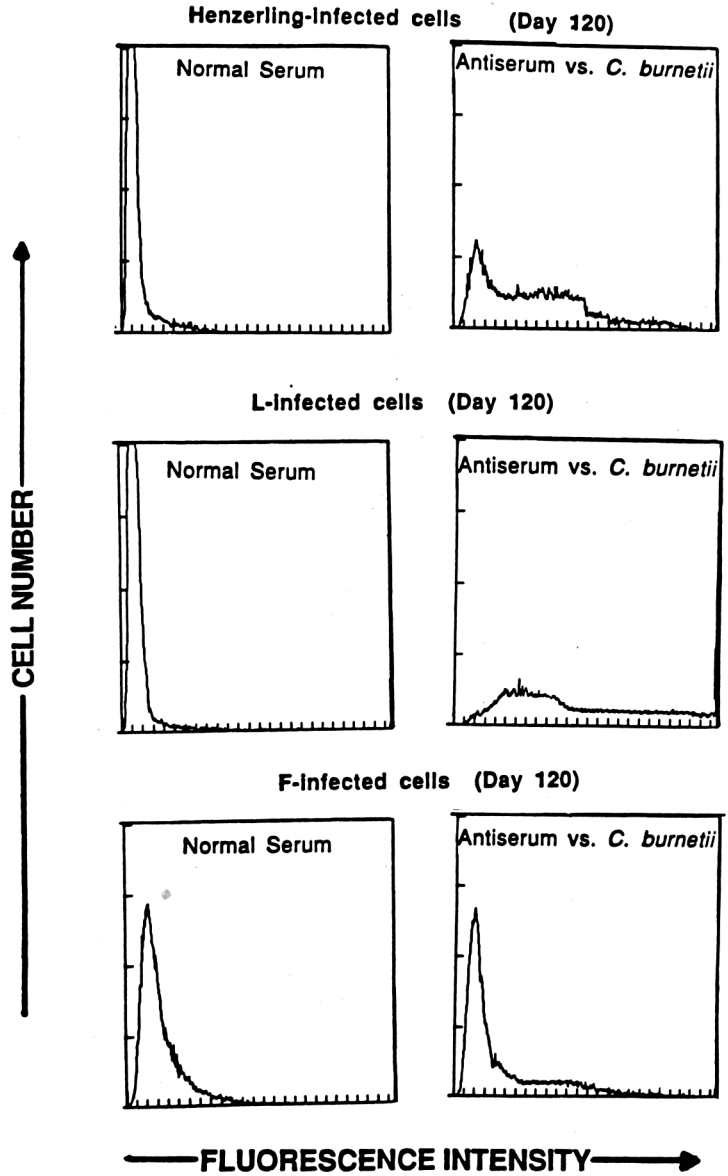

Fig. 7

Histogram of long-term infected cells (for 120 days) examined by flow cytometry Infected cells treated with normal and serum to *C. burnetii* are shown. Henzerling-infected cells exhibited the greatest amount of Texas Red-anti-IgG fluorescence followed by L-infected cells. F-infected cells showed the least amount of fluorescence.

cells had several highly infected vacuoles, while cells infected with the other four isolates usually contained a single highly infected vacuole.

Coxiella burnetii antigens are expressed on the host cell surfaces. Depending on the infecting isolate, different amounts of antigen are inserted into the host cell membrane which may account in part for the different disease manifestations. The presence of antigens on the host cell surface may allow infected cells to be detected by the immune system, leading to their lysis; released parasites may then be rendered vulnerable to destruction by activated macrophages. Since acute isolates cause the insertion of the greatest amount of antigen into the host membrane, they may readily signal the immune system and result in their elimination. If this scheme is correct, an individual with defective cell mediated immunity would not be able to deal with a *C. burnetii* infection, since release of the parasites from infected cells would spread the organisms to other areas. Because chronic isolate infection caused the insertion of fewer antigens, they may therefore be less visible to the immune system and persist.

This *in vitro* model has proven to be useful in allowing us to compare some of the morphological characteristics of *C. burnetii*. The findings of this study have revealed some biological differences among the isolates, specifically among the three major groups, which may shed light on *in vivo* disease manifestations.

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