

INFECTION OF CELL CULTURES BY CAMPYLOBACTER SPUTORUM SUBSPECIES MUCOSALIS

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Intracellular vibrioid bacteria are a constant feature of the lesions of porcine intestinal adenomatosis (PIA) and from such tissues Campylobacter sputorum subspecies mucosalis may often be recovered generally in large numbers. Despite the constant presence of bacterial bodies within the abnormal cells, the part played by bacteria in the induction of the lesion remains unknown. Experimental evaluation of the role of individual infectious agents in the development of the condition has not proved easy and this situation has been compounded by the difficulty most workers have experienced in trying to transmit the disease even when diseased mucosa has been used as inoculum. Such a refractory infectious nature is in contrast to some field experience of the disease and no acceptable explanation for this difference is available.

In the absence of a satisfactory experimental system using live animals, an attempt was made to assess the ability of C. sputorum ss mucosalis to survive in both continuous and primary cell culture derived from different species and to assess whether this was associated with intracellular multiplication of the bacteria.

Initial observations concentrated on assessing the ability of mucosalis serotype A to attach to a variety of cell types, principally derived from pig, fowl, ox, sheep, dog and primate sources. Young viable cultures of mucosalis attached rapidly to the surface of all the pig derived cell line monolayers (primary pig kidney, PPK; pig kidney, PK and PKpi); attachment to some avian (chick embryo fibroblast, CEF) and some primate cell lines (Vero and monkey, LLCMK2) could not be demonstrated using the light microscope, other cell lines (bovine kidney, BK, BKpi; hamster, BHK and Hela cells) showed intermediate levels of attachment.

Peripheral attachment of mucosalis reached its peak in appropriate cells within some 6 hours of infection and thereafter declined until by 12 hours only a small percentage of cells showed adherent bacteria and these were no longer apparent by 24 hours post-infection. Attachment is influenced by the culture temperature prior to and subsequent to inoculation and either raised (41°C) or lowered (22°C) temperature reduced attachment and the motility of cultures. Formalin-killed bacteria do not adhere and air-killed organisms show a different pattern of attachment. Attachment can be blocked by hyperimmune mucosalis antiserum.

Cell and antibiotic free tissue culture medium (MEM) does not support the growth of mucosalis unless the atmosphere is altered. MEM media containing $7.0 \log_{10}$ mucosalis/ml fail to yield viable bacteria after 12 hours aerobic incubation at 37°C. In contrast, cell cultures in MEM of certain lines persistently yield viable bacteria from the supernatant fluid. Such persistent infection appears to be associated with those cell lines to which mucosalis attach and cell lines to which mucosalis do not attach do not yield the organism for any length of time.

Cell lines to which mucosalis attach show intracellular bacterial forms, often within phagosomes, by 24 hours at which stage bacteria are largely absent from the cell surface. Such bacteria within the cells retain their characteristic vibrioid morphology. The number of intracellular bacteria vary between cell lines being most prominent in strongly adherent cell types. These observations have allowed a technique to be developed for demonstrating intracellular (IC)

and extracellular (EC) bacteria by fluorescent antibody (FA). After 48 hours IC bacteria lose their typical appearance in Giemsa-stained smears but their presence can be demonstrated in increasing numbers in the cell cytoplasm by either FA or electron microscopy in cell lines that resist destruction. The cell parasitism is in many cases associated with marked disturbance in the appearance and viability of the cell cultures.

The development of appropriate infection procedures for tissue culture may provide an alternative approach to the investigation of the PIA Complex should the disease remain difficult to reproduce experimentally.

Selected references: Rowland, A.C. and Lawson, G.H.K. (1974). Res. vet. Sci. 17:323; Love, R.J., Love, D.N. and Edwards, M.J. (1977). Vet. Rec. 100:65.