Survival of *Bordetella pertussis* in Tissue Culture of Animal Cells

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Summary

Wild type *Bordetella pertussis*, Tohama strain was used in infection assays of four continuous cell lines human Foreskin, white mouse, monkey and bovine kidney cells. The result depended on the origin of cell line. One week after infection human Foreskin cell showed cytopathic effect and the bacteria were alive but in mouse cell just two days after infection the bacteria were beginning to die. Mean but in monkey and bovine cell lines the bacteria strain was able to persist within the host cells and a symbiotic relationship between cell and bacteria was seen.

Key words: *Bordetella pertussis*, pertussis, cell culture, cell line, intracellular bordetella

Introduction

Since the production and administration of the first vaccines against pertussis has been generally thought that animal cells had no receptor for *Bordetella pertussis* (Manclark *et al* 1993, Mirchamsy 1997). Until recently, *B.pertussis* was considered as an obligate human pathogen because of there was no animal reservoir for the disease (Bemis & Burns 1994) it would be possible to completely eliminate it (Mirchamsy 1997). But even after carrying out Expanding Program of Immunization

(EPI) re-infection of pertussis among adults was reported (Rosenthal *et al* 1995, Ruuskanen *et al* 1991). Because of lack of natural exposure to the disease in childhood and waning immunity after immunization, adult population (>20 years) is susceptible to the infection, so atypical and mild pertussis illness among them is considered as an important reservoir for the organism (Shefer *et al* 1995). Despite the previous theory that except for infected children pertussis bacteria has no any reservoir, some recent data suggested that *B.pertussis* can invade and survive in several types of eukaryotic cells, such as CHO and HeLa (Crawford & Fishel 1959, Eeanowich *et al* 1989a). In this paper we; in which the use of four continuos human and animals cell lines: Razi human diploid Forskin cell (line FSC2), white mouse originated cell (BSR), Rhesus monkey kidney cell (Vero) and Razi calf kidney cell (BK) as mean of infection assay; evaluate the invasive ability of *B.pertussis*.

Materials and Methods

Bacterial strain. The wild type *B.pertussis*, Tohama strain was used in this study.

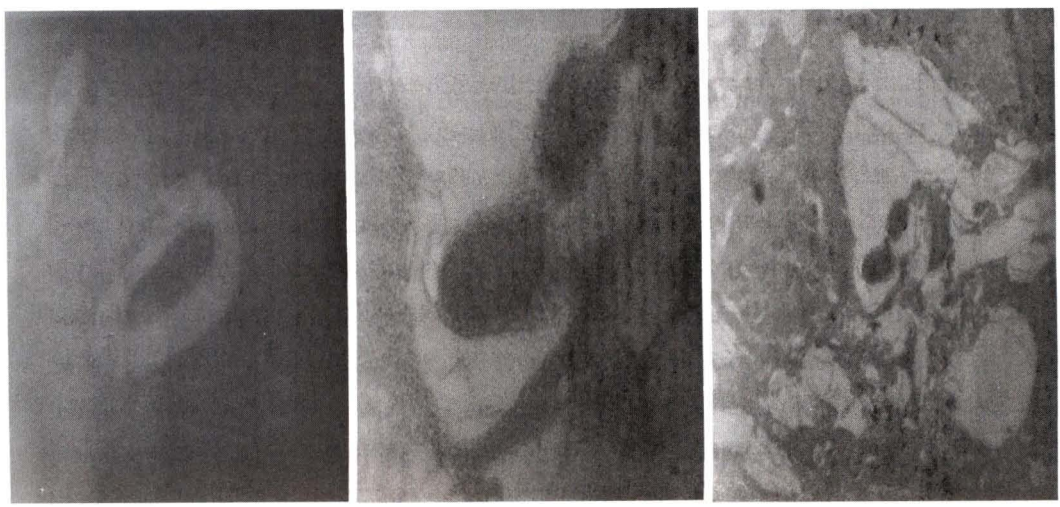
Invasion assay. B.K. cells (a Razi Institute calf kidney cell line) were used in invasion assays as describe here. Tissue culture flasks (10 Roux bottles) were seeded with approximately 10^8 *B.pertussis* per bottle and incubated at 37°C. After 5h adsorption, the medium replaced with amount Dubleccos Eagle minimal essential medium (MEM) containing 100mg/ml gentamicin and incubated at 37°C for 10h to destroy remaining extracellular bacteria according to Hendrik *et al* (1994). Next, the medium was replaced with MEM containing only 10mg/ml gentamicin to prevention the side effects of the antibiotic on the epithelial cells. The cultures were observed for normal growth up to 16 days. At the end of examination cells were removed from the bottle and divided in to two parts one for electron microscopy study and the other for transferring to specific culture media, Bordet-Gengou agar and Verwey (Mirchamsy 1997). The same invasion processes were used for the other cell lines. In all cases, some tissue culture flasks were set up as control for comparing bacterial growth.

Transmission electron microscopy (TEM). TEM was used for detection and confirm intracellular location of the bacteria (Ewanowich *et al* 1989b). Briefly, the harvested

cells were fixed by 3% glutaraldehyde and 2% osmium tetroxide then centrifuged at 2000 rpm to obtained a cellular plug. The plugs were blocked in 2% Nobel agar (Sigma). After dehydration and infusion the resin, the plug were blocked at BEEM (Best Equipment Electron Microscope) capsules. The serial thin sections (400Å) were prepared and stained with Uranyl acetate and lead citrate. Photographs were taken with a Philips 400 electron microscope (WHh 100 Voltage).

Results and Discussion

Intracellular survival of *B.pertussis* was analyzed by TEM. During the first week, CPE was observed in human Foreskin cells and the bacteria were alive, whereas after 2 days the number of viable *B.pertussis* started to decrease in mouse cells until no viable bacteria were detected after 16 days, the cell line was healthy and free of bacteria. At 3 days postinfection, the strain of *B.pertussis* had survived intracellulary in B.K cells (Fig. 1).



a b c

Figure 1. *TEM demonstrating intracellular B.pertussis strain Tohama in B.K cell lines. a) Magnification, ×75,000. b) Magnification, ×45,000. c) Magnification, ×21,000*

Further more, the growth of *B.pertussis* was observed 3 days after transferring of the contaminated cells in to the specific culture media. Similar result was obtained with the Vero cell line (Fig. 2).

a b c

Figure 2. *TEM demonstrating intracellular B.pertussis strain Tohama in Vero cell lines. a) Magnification, ×75,000. b) Magnification, ×27,000. c) Magnification, ×45,000*

Today, at the end of 20th century, *B.pertussis* still causes a common highly infectious respiratory disease that predominantly affects children. As many as 60 million cases with more than half a million deaths (Muller *et al* 1986) and 1 million defective children occur annually. Since 1970, immunologists pay attention to the production of high quality pertussis antitoxin. They also believed that there was no animal reservoir for pertussis in nature (Bemis & Burns 1994) and the only way to transfer the disease was direct contact between people. Therefor by immunization it should be possible to eradicate the disease (Manclark & Cowell 1993). More than 25 years have passed and despite EPI programs, there are many reports of outbreak of pertussis among adults (Rosenthal *et al* 1995) and children (Kwantes *et al* 1983) in developing countries. The *in vitro* evidence showed that *B.pertussis* can be found in

animal cell culture it suspect that this phenomena may occur in nature and so, animal plays a reservoir role. Epidemiological surveys based on recent reports have shown that most cases of disease occur in areas where domestic animals are in contact with human. This reverts the eradication of the disease (Bemis & Burens 1994). Despite the disease is not specific for children these observations indicate that we should revise our ideas about pertussis epidemiology, and can scilicet the possibility of animal reservoirs, the role of host cell in the preservation, replication, and virulence of *B.pertussis*. In this study we demonstrate that the *B.pertussis* strain Tohama has the capacity to invade and to persist in two animal cell lines. Further examination with respect to the occurrence of this phenomenon *in vivo* in animals, which considered as reservoir is required.

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