MOLECULAR ANALYSIS OF HOST CELLS RESPONSE AGAINST INFECTION OF VIRULENT STRAINS *LISTERIA MONOCYTOGENES*

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PENGHARGAAN

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5.2 Enhanced Executive Summary

5.2.1 MICROSCOPIC EXAMINATION OF ACTIN HOST CELLS REARRANGEMENT FOLLOWING INFECTION OF *LISTERIA MONOCYTOGENES*

ABSTRACT

Listeria monocytogenes is a foodborne pathogenic bacteria causing severe systemic infection in susceptible individuals. This facultative intracellular pathogen infects host tissues using their flagella actin. *Listeria monocytogenes* propelled from one cell to another through the assembly of the flagella actin with the host cells.The present study aims to examine the rearrangement of host cells cytoskeleton actin upon infection of *Listeria monocytogenes*. Human colon carcinoma (HCT 116) cell lines were infected with *Listeria monocytogenes* strains. Cells were stained with alexa fluor 635 phalloidin prior to observation by confocal laser scanning microscopy (CLSM) to examine cellular changes of the host cells upon infection with the bacteria. The infected cells showed actin rearrangement from filamentous network to aggregation features. The infected cells also showed reduced membrane ruffling. The present study demonstrated interaction of host cells actin with *Listeria monocytogenes*. Confocal laser scanning microscope (CLSM) examination revealed the cytoskeleton actin rearrangement in the infected host cells.

Keywords: *Listeria monocytogenes*, cytoskeleton actin, Confocal laser scanning microscope (CLSM)

INTRODUCTION

Listeria monocytogenes is a Gram-positive facultative intracellular pathogen that causes food-borne infections in animals and humans. The ability of *Listeria monocytogenes* to cause disease correlates with its capacity to cross barriers of human host [1]. Following ingestion contaminated foods; *Listeria monocytogenes* crosses the intestinal barrier by invading the intestinal epithelium thereby gaining access to internal organs. The disease caused by this bacterium, was manifested as encephalitis, meningitis, gastroenteritis, septicaemia, mother to fetus infection and resulting in death.

Bacterial protein, Act A responsible in the intracellular cell to cell spread of listeria and polymerization of it actin from cytoplasm [2]. Act A protein allows intracellular movement of the bacteria into host cells and promotes cell to cell spreading by activation of host cells protein complex [1]. Interaction of the cellular receptors E-chaderin and Met with the Listeria monocytogenes surface proteins InlA and Inl B, is required for intracellular invasion. This interaction activates signaling cascade, leading to actin rearrangements responsible for plasma membrane remodeling and bacterial uptake into host cells [4].

The present study aims to examine rearrangement of host cells cytoskeleton actin upon infection of *Listeria monocytogenes*.

MATERIALS AND METHODS

Cell culture and invasion assay

Confluent Human Colon Adenocarcinoma Cell Line (HCT 116) cells monolayer were trypsinized and adjusted to a concentration of 10x105 cells ml-1 in antibiotics free culture medium. One ml cell suspension was dispensed into each well of inoculating 4 –chamber slides (Lab tek Chamber slide ,Nunc, USA) and incubated at 37 ºC in a humidified atmosphere of 5% Co2 in air, for 24 hours, to obtain a semiconfluent monolayer. Biological Triplicates were done prior to infection, cells were incubated in serum, antibiotics free medium for one hour. The monolayer was washed twice in 1 ml of sterile PBS to remove fetal bovine serum (FBS) which inhibits bacterial uptake. The semiconfluent monolayer were inoculated with bacterial suspension adjusted to obtain multiplicity of infection (MOI) 10 bacteria per cells. After one hour, the monolayers were washed twice with sterile PBS and covered with 1ml prewarmed fresh medium supplemented with penicillin/streptomycin and gentamycin a bactericidal concentration (50 µg) to kill extracellular bacteria. After four hour incubation, well chamber plate were washed twice with PBS. The monolayer were washed 3 times with PBS to remove medium, then later replaced with complete medium.

Fluorescence staining of bacteria and host cytoskeleton confocal microscopy observation

After four hour incubation, the 4 well chamber plate were fixed with 4% paraformaldehyde, followed by permeabilization with 0.2% Triton X-100 (Sigma). The cells were then added with quenching solution to block free aldehyde group. The monolayer cells were incubated with primary antibody. After 30 minutes incubation, the primary antibody was washed with blocking solution. Secondary antibody was added with the cytoskeletal probe Alexa fluor 488 phalloidin. After washing step, the chamber was removed, and the slide mounted with prolonged gold antifade reagent. After 24 hour stored in cold temperature, the slide were viewed using confocal microscope. Fluorescent preparation were observed in a confocal fluorscence imaging, using FluoView FV1200 and the picture recorded on a flat-screen black and white monitor with high linearity.

RESULTS

Fluorescence staining of Human colon adenocarcinoma cell line , HCT 116 revealed that actin rearrangement could be observed 4 hours post infection in the cell lines. Observation in figure1 displays the successive steps of intracellular infection. Using Confocal laser scanning microscopy (CLSM) , we demonstrate the formation filaments of actin stress fiber (A). Membrane ruffling as a results of actin rearrangement can be observed as formation of barbed end or protusion of the host actin (B). Host cells and bacterial actin polymerization can be observed as rearrangement of F-actin in the comet tails (C). Generation of membrane vacuole indicate the engulfment of bacteria for intracytoplasmic infection (D).