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Pathogenic and Virulence Mechanisms of Intracellular Bacteria: An Insight in Designing Appropriate Therapeutic Approach

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SUMMARY
Intracellular bacteria can survive and multiply inside phagocytes. Successful intracellular bacterial pathogens have evolved complex and efficient methods to overcome innate and adaptive immune responses. There are several pathogenic and virulence factors that are used by these pathogens to evade the immune system. Pathogenic intracellular bacteria prevent phagosome lysosome fusion, impair the respiratory burst and also inhibit chemotaxis and interfere with macrophage activation. They can also inhibit antigen presentation, destruct phagosome membrane, resist antibacterial peptides and also parasitize non-professional cells. Secreted proteins and siderophores also assist the survival and multiplication of intracellular bacteria within host cells. The complement and adaptive immunity may be paralyzed for successful survival of some of these bacteria. Some intracellular bacteria carry virulence genes that regulate host factors. Understanding the pathogenic mechanisms and the virulence factors of intracellular bacteria are crucial in underlying the pathogenesis and in designing appropriate therapeutic approach. Hence, research should be conducted at molecular and genetic level to further characterize and understand the pathogenic and virulence factors associated with intracellular bacteria.
Keywords: Evasion, Innate and Adaptive Immunity, Intracellular Bacteria, Pathogenic and Virulence Factors

1. INTRODUCTION

The vigilance of mammalian defense mechanisms is very critical for successful survival of animals and human beings in an environment where pathogenic microorganisms are ubiquitous. Resistance of the host to these pathogens requires the careful orchestration of a series of molecular, humoral and cellular interactions. One of the mechanisms of resistance to pathogens is conferred by the innate immunity, which is the first line of defense against pathogens. When this arm of the immune system fails to provide protection, the adaptive and more specific immune system is activated to provide protection (Roth, 1988).

However, successful intracellular bacterial pathogens *(Mycobacteria, Brucella, Salmonella, ,Listeria, Shigella, Rickeusia, Chlamydia, Legionella* and *Niesseria)* have evolved complex and efficient methods to overcome and evade innate and adaptive immune responses. The various evasion strategies used by these bacterial pathogens are numerous and complex (Finlay and McFadden, 2006).

Generally, intracellular bacteria employ several different strategies to ensure their survival within macrophages and neutrophils (Tizard, 1996; Todar, 2002). These bacteria survive and grow within macrophages by inhibiting phagolysosomal fusion and by impairing the respiratory burst mechanism. Others may also inhibit the migration of defense cells to the sites of infection (Tizard, 1996). Recently, it has been determined that the intracellular activities of *Salmonella enter/ca* prevent protein biosynthesis and this affect the capacity of dendritic cells to present antigens to T-cells (Cheminay *et al.,* 2005). Moreover, intracellular bacteria can minimize their interaction with cellular defenses of the host by parasitizing non-professional cells. Early escape from the phagosome vacuoles is known to be a clever strategy utilized by intracellular pathogens to avoid their destruction within phagosmomes (Todar, 2002). Since iron is an absolute requirement for the growth of almost all bacteria, these invasive intracellular bacteria always compete for host iron and utilize their sidrophores to remove iron from iron binding proteins of the host (Hirsh and Zee, 1999).

Furthermore, most intracellualr pathogens survive and replicate within host tissues by paralyzing complement and adaptive immunity. The virulence genes of bacteria and secreted proteins significantly assist the survival of intracellular pathogens within host cells (Hirsh and Zee, 1999; Gyles *et al.,* 2004). Therefore, the overall objective of this review paper is to insight and describe the pathogenic and virulence factors utilized by intracellular bacteria to evade especially the innate immunity.

2. PATHOGENIC MECHANISMS OF INTRACELLULAR BACTERIA

The successes of pathogens to exist within host tissues are directly dependent on their ability to mount an effective anti-immune response (Finlay and McFadden, 2006). In general, bacteria that can survive and multiply inside phagocytes and other cells of the host are considered as intracellular bacteria. Intracellular bacteria survive inside phagocytes by virtue of their virulence mechanisms which interfere with the bactericidal activities of host cells (Todar, 2002).

2.1. Mechanisms of Evading Innate Immunity

2.1.1. Prevention of Phagosome Lysosome Fusion

Once foreign particles are attached to the membrane of neutrophils and macrophages, the primary granules or lysosomes migrate through the cytoplasm and fuse with the phagosome membrane to create a digestive vacuole called phagolysosome (Tizard, 1996). Hence, killing and digestion of engulfed microbes takes place within the phagolysosomes by microbicidal substances like lysozyme, cationic proteins, proteases and peroxidases (Todar, 2002). However, some bacteria, such as *Brucella abortus* and *Listeria monocytogenes* are resistant to the lethal effects of the phagolysosomal microbicidal constituents (Tizard, 1996).

Recent reports have indicated that cholesterol dependent association of tryptophanaspartate containing coat protein (TACO) plays a crucial role in the entry/or survival of *Mycobacterium tuberculosis* within human macrophages (Anand and Kaul, 2005). The structures and biologic functions of the glycolipid cell wall of *Mycobacteria* have been investigated and found to assist the survival of *Mycobacteria* within macrophages. The glycolipid, especially the suipholipids are known to contribute to the pathogenic effects of *Mycobacteria* by preventing phagosome lysosome fusion and, thus, prevent lysis of bacteria within phagocytes (Gyles *et al.,* 2004). The cord factor (suipholipid) is especially the most virulent factor (Hirsh and Zee, 1999).

The eukaryotic like serine/threonine protein kinase G (Pkn G) from pathogenic *Mycobacteria* is secreted into the cytosol of infected macrophages and inhibits phagosome lysosome fusion and mediate intracellular survival and multiplications of these pathogens within host cells (Wolburger *et al.,* 2004). Moreover, *Mycobacteria* have the ability to prevent acidification of phagosome and thereby decrease the release of bactericidal factors (Abigail and Diexie, 2002). However, a lack of proton ATPase in the vascular membranes surrounding *Mycobacteria* containing phagosomes might be responsible for the altered biochemical composition and lack of phagosomal acidification (Sturrggill-Koszycki *et al.,* 1994).

Furthermore, *Brucella* is capable of surviving and multiplying inside macrophages by inhibiting phagolysosome fusion (Ko and Splitter, 2003). Adenine and 5-guanosine monophosphate of *Brucella* inhibit phagolysosome fusion in neutrophils (Hirsh and Zee, 1999). Normally, maturation of newly formed phagosome to fuse with lysosomes is a fast process. The delayed maturation observed for *Brucella* phagosomes is very important to prevent early digestion and allow the bacteria to express new genes necessary for intracellular survival (Ko and Splitter, 2003). *Neisseria gonorrhoea* also inhibits phagolysosome formation by their outer membrane proteins (Todar, 2002). Similarly, *Chlamydia* prevents phagolysosomal fusion and acidification of the endosome so that these pathogens can multiply in the cytoplasm of macrophages (Gyles *et al.,* 2004). Prevention of phagosome lysosome fusion is also employed by *Legionella* and *Salmonella* (Todar, 2002). *Legionella pneumophilia* c ell s urface m odify the m embranes o ft he p hagosomes, thus p reventing their merger with lysosomal granules. In *Legionella,* a single gene is responsible for the inhibition of phagolysosomal fusion (Todar, 2002). Interestingly, *Salmonella* are also able to inhibit fusion of phagosomes with lysosomes by *Salmonella* Pathogenicity Island 2 (SPI2) mediated processes (Vazguez-Torres and Fang, 2001).

2.1.2. Inzpairnieiit of the Respiratory Burst

Within seconds of binding to foreign particles by their Fc or mannose receptors, neutrophils and macrophages increase their oxygen uptake for subsequent production of lethal oxidants as antibacterial agents. This mechanism is called the respiratory burst (Todar. 2002). These receptors activate a membrane bound NADPH oxidase that reduces oxygen molecule (O_2) to superoxide (O_2). Then O_2 can be reduced to hydroxyl radical (01-U) or dismutated to hydrogen peroxide (H2 O_2) by superoxide dismutase (SOD). O_2, 01-U and H_2O_2 are activated oxygen species that are potent oxidizing agents in biological systems (Majno and Joris, 2004). Myeloperoxidase (MPO) uses H_2O_2generated during the respiratory burst to catalyze halogenation, mainly chlorination of phagocytosed microbes. Such halogenations are potent mechanisms for killing cells. In this way, when the NADPH oxidase and MPO systems are operating in concert, a series of reactions leading to lethal oxygenation and halogenation of engulfed microbes occurs (Todar, 2002).

One of the mycobacterial constituents, the cord factor (dimycolyl trehalose), disrupt mitochondria which then leads to disturbance in cellular respiration and generation of free radicals to kill microbes. In addition, sulpholipids (sulfatides), phospholipids and phosphatidyl inositolmannoside may also prevent the respiratory burst and may interfere with the function of reactive oxygen intermediates following ingestion of tubercle bacilli by macrophages (Hirsh and Zee, 1999). Macrophages efficiently kill *Mycobacteria* by oxygen derived metabolites like H202 and hydroxyl radicals (Gyles *et al.,* 2004). However. SOD produced and released by several mycobacterial pathogens could protect the organisms from the toxic effects of reactive oxygen radicals generated during the oxidative respiratory burst (Andersen *et al.,* 1991).

Intracellular survival of *Brucella* in macrophages and to a lesser extent in neutrophils is also mediated by suppressed MPO H_2O_2 halide system (Hirsh and Zee, 1999). Moreover, SOD and catalase produced by *Bruce/la* may avoid the destruction of this pathogen by oxidative respiratory burst mechanism (Hirsh and Zee, 1999). The resistance of *B. aborius* to phagocyte mediated killing has been also correlated with the purative release of nucleotides that compromise the neutrophil function (Roth, 1988). *Neisseria gonorrhoea* possibly reduce

respiratory burst by its outer membrane protein (Todar, 2002). SOD has been suggested to protect *Listeria monocytogenes* against killing by phagocytes (Roth, 1988). Some intracellular bacteria utilize a unique mechanism to escape the respiratory burst. For example. intracellular *Salmonella,* which reside within a specialized membrane compartment called the *Salmonella* containing vacuole (SCV) in macrophages, use a type 3 secretion system (T3SS), the *Salmonella* Pathogenicity Island 2 (SPI2), to mediate protection from reactive nitrogen intermediates (Roth, 1988). The presence of this unique structure avoids co-localization of *Salmonella* with harmful host enzymes. Similarly, *Salmonella* SPI2 also evade phagocyte NADPH oxidase mediated killing and thereby ensure their survival intracellularly within macrophages (Finlay and McFadden, 2006).

2.1.3. Inhibition of hemotaxis and Interference with Macrophage Activation

Leukocytes migrate towards infected tissues in response to chemoattractants to effect clearance. However, some intracellular bacteria inhibit the migration of leukocytes, particularly neutrophils to the site of infection. Fore example, defective neutrophil chemotaxis during lepromatous leprosy has been confirmed (Roth, 1988). The cell wall component of *Mycobacteria,* trehalose-6, 6 dimycolate, inhibits chemotaxis and is also leukotoxic (Gyles *et al.,* 2004). Interferon gamma (IFN-y) and other cytokines produced by CD4 T-cells are essential for activating macrophages. However, *Mycobacteria* produce factors that interfere with CD4 T-cell activation and release of IFN-y (Abigail and Diexie, 2002). Then this indirectly interferes with macrophage activation, thus, ensuring the survival of *Mycobacieria* in macrophages. Even within activated macrophages, *M tuberculosis* has been shown to enhance its survival and persistence in the host by up-regulating the expression of isocitrate lysase. an enzyme involved in fatty acid metabolism (Gyles *ci at.,* 2004). Interestingly. Plasminogen activator protease (Pla) in *Yersinia pestis* prevents chemotaxis of polymorphonuclear cells (PMNs) to the site of infection (Abigail and Diexie, 2002).

2.1.3. Inhibition ofAntigen Presentation by Dendritic Cells

The main function of dendritic cells (DC) is antigen presentation. DCs can take up diverse arrays of antigens and present processed peptides that bound to major histocompatibility complex (MHC) class I and II to T-cells (Groux *et al*,. 2004). Recently, it has been determined that the intracellular activities of S. *enterica* prevent protein biosynthesis and degradation in intracellular vesicles and this affect the capacity of DC to present antigens (Ags) to T-cells. Furthermore, intracellular *Salmonella* increases the expression levels of inducible nitrogen oxide synthase (iNOS) and production of nitrogen oxide by DC. This, therefore, suppress T cell proliferation after Ags are presented properly. Moreover, the intracellular activities of SPI2 have resulted in a reduction of Ag presentation on MHC class II molecules. Therefore, the direct inhibition of Ag processing and/or presentation by intracellular *Salmonella* via a defined virulence system represents a novel strategy of immune escape for this pathogen (Cheminay *et al., 2005).*

2.1.4. Lysis of Phagosome Membrane and Escape from the Phagosome

Early escape from the phagosome vacuoles (phagosome) is known to be a clever strategy utilized by intracellular pathogens to avoid their destruction within phagosomes (Todar, 2002). Once entered into the cells, *L. monocytogenes* relies on its cholesterol binding hemolysin (Listeriolysin 0) and a phosphatidyl inositol dependent phospholipase C to lyse the phagosome membrane and escape into the cytoplasm of macrophages (Hirsh and Zee, 1999; Decatur and Portnoy, 2000). Similarly, a bacterial enzyme phospholipase A, is responsible for dissolution of the phagosome membrane and discharge of rickettsial organisms (Todar. 2002). Recently, a gene has been detected in *M. tuberculosis* genome sequence that has sequence homology to hernolysin of *L. monocytogenes* that allow this microbe to escape from a phagocytic vesicle (Abigail and Diexie, 2002). As well as, *Shigella* lyses the phagosornaJ vacuole and induces cytoskeletal actin polymerization for the purpose of intracellular movement and cell to cell spread (Todar, 2002).

2.1.5. Resisting Antibacterial Peptides

Peptides that intercalate into bacterial membranes have been known to create pores via which essential interior molecules escapes (Abigail and Diexie, 2002). These peptides are defensins and cathelicidins (Finlay and McFadden, 2006). Defensins first transit the peptidoglycan and outer membranes (in the case of gram negative bacteria) to reach the vulnerable cytoplasmic membrane. Some intracellular pathogens, however, alter their surface structure to decrease insertion of anti-bacterial peptides into their cell membrane. They encode transport systems that remove these peptides and they can also secrete proteases that degrade these peptides before they can reach the cytoplasmic membranes. For example, *Salmonella* secrete proleases to destruct antimicrobial peptides and are resistant to defensins (Finlay and McFadden, 2006). *Shigella* also suppresses the expression of epithelial antibacterial peptides by enterocytes so that the microbe will survive in the gut and subsequently infect microfold cells (M cells) (Michael, 2002).

2.1.7. Secreted Proteins

Pathogenic tubercle bacilli and other *Mycobacteria* contain several proteins and protein complexes that may enhance the development of cellular immunity. Of particular interest are secreted proteins in the antigen 85 complexes (Kaufmann *et al.,* 1990). However. fibronectin and extracellular matrix component of tissues binds to antigen *85* complexes and then prevent or reduce the release of large amount of this antigen. This, therefore,

impairs the development of cellular immunity to tubercie bacilli (Abou-Zeid *et al.,* 1998).

Large amount of heat shock proteins (HSPs) are secreted by *Salmonella* that favor the survival of this microbe within macrophages (Gyles *et al.,* 2004). Stress proteins or HSPs have been also demonstrated in *Brucella* (Hirsh and Zee, 1999). An elevatçd synthesis of HSPs in response to changes in physiological conditions within the intracytoplasmic vacuole (phagolysosome) may protect *Mycobacteria* and other intracellular pathogens from hydrolytic enzymes, reactive oxygen free radicals and MPO killing mechanisms (Andersen *et al*.. 1 991).

2.1.8. Production of Iron-Chelators (Siderophores)

Siderophores are iron carrying molecules (catechols or hydroxamates) of bacterial origin. They function in the solubilization and transport of ferric irons. Siderophores synthesized by bacteria are exported into host fluids, where they capture iron associated with iron-binding proteins of the host such as with ferritin, transferrin and lactoferrin. Then siderophores return to the bacteria through specific outer membrane receptor proteins produced in response to low concentrations of iron (Finkelstein *et al.,* 1983). As iron is an absolute requirement for bacterial growth, invasive intracellular bacteria always compete for host iron and utilize their siderophores to remove iron from iron binding proteins of the host (Hirsh and Zee, 1999). *Yersinia,* however, assimilates iron by siderophore-independent mechanisms and use hemin as the sole source of iron for multiplication (Gyles *et al.,* 2004). S. Typhimurium produces enterochelin (enterobactin), a phenolate siderophore, for capturing iron (Gyles *et al*., 2004). *Mycobacteria* also use iron chelators (exochelins and mycobactin) that are responsible for scavenging iron for intracellular growth (Hirsh and Zee, 1999).

2.1.9. Parasitizing Non-professional Phagocytic Cells

One mechanism by which bacteria can minimize their interaction with cellular defenses of the host is by parasitizing non-professional cells. This mode of pathogenesis is well recognized for *Rickeitsia* and *Chiarnydia* that prefer to infect endothelial and epithelial cells. *Ricketisia* adheres and internalize to the endothelial cells with the aid of an outer membrane proteins (Roth, 1988). *Shigelta, L. monocytogenes* and *B. abortus* also use this strategy when they invade non-professional phagocytic cells (Roth, 1988). *Bruce/la* can infect non-professional cells such as the trophoblasts of pregnant ruminant animals *in vivo*. Moreover, virulence factors of *Bruce/ta* assist colonization of the placenta with further replication (Gyles *et al*.. 2004). Recent evidences have also indicated that *Brucella* is known to migrate through the autophagic pathway before hoarding in the endoplasmic reticulum, where *Bruce/la* multiplication has occurred (Gyles *et al,*. 2004).

Salmonella has been shown to adhere and invade the epithelial cell layer lining of the intestine by SPI-1 and fimbriae. Before adhering to the epithelial cells, *Salmonella* initially parasitizes M cells (Gyles *et al.,* 2004). In addition, *Yersinia pesils* has an outer membrane protein called Invasin (mv), which facilitates adhesion and entry into the epithelial and human endothelial cells, which then multiplies and disseminates from the site of infection to lymph nodes or other organs with the help of Pla (Hirsh and Zee. 1999; Gyles *et al*.. 2004). Invasion and adhesion of *Listeria* to the host tissues require a unique 80 kilodalton secreted surface protein known as internal in that mediates invasion. Then internalin binds to an unidentified receptor on mammalian epithelial cells which results in internalization of *Listeria* (Gyles *et al.,* 2004).

2.2. Evading the Complement

Capsule is a network of polymers that covers the surface of bacteria (Abigail and Diexie, 2002). The sialic acid of bacterial capsule of *Neisseria gonorrhea* prevents the activation of the alternative pathway by preventing the formation of C3 convertase that split the third component (C3) of the complement system into C3a and C3b. In general, this prevents the activities of the complement system so that *Neisseria gonorrhea* can evade phagocytes (Tizard, 1996; Roitt, 1997). *S.* Typhimurium has a gene called resistance to complement killing (RCK) that confers resistance to complement mediated lysis by preventing the insertion of C9 (the last component of membrane attack complex) into bacterial outer membrane (Tizard, 1996; Abigail and Diexie, 2002). In *)'ersinia, Yersinia* adherence A (Yad A) also prevents complement activation (Gyles *et al.,* 2004). Furthermore, gram positive intracellular pathogens have a thick peptidoglycan layer that prevents the insertion of lytic C5b-9 membrane attack complex into the bacterial cell membrane (Roitt, 1997). In general, lipopolysaccharides (LPS) and capsule components of bacteria may prevent the lytic activities of complement (Roth, 1988).

2.3. Evading Adaptive Immunity

2.3.1. Local Cytokine Paralysis

The cytokine interleukin 8 (IL-8) is a potent chemoattractant and activator of PMNs and is secreted by gingival epithelial cells in response to the components of normal oral flora. In contrast, *Poiphyromonas gingivalis* was found to strongly inhibit IL-8 secretion from gingival epithelial cells. This inhibition was associated with a decrease in the expression of messenger ribonucleic acid for IL-8. Proteases and LPS of this microbe are known virulence factors to inhibit IL-8 secretion. Therefore, invasion-dependent destruction of the gingival IL-8 gradient at sites of *P. gingivalis* colonization, severely impair mucosal defense and represents a novel

mechanism for bacterial colonization of host tissues (Darveau *et al.,* 1998).

2.3.2. Evading Humoral Immunity

Mainly the outer membrane proteins of pathogens are the targets of antibodies (Todar, 2002). However, an effective bacterial strategy for evading host's antibody (Ab) response is to change the surface Ags that are recognized by Abs so that Abs no longer binds to antigenic surfaces. *Salmonella* and *Neisseria* change their surface coat to mimic host tissue so that Abs could not recognize and destroy them (Abigail and Diexie, 2002).

2.3.3. Evading T-cell Immunity

Microbial pathogens have been selected for the capacity to evade or manipulate host responses in order to survive after infection. *Chiamydia* can escape T-cell immune recognition by degrading host transcription factors required for MHC. A chiamydial protease-like activity factor (CPAF) secreted into the host cell cytosol represents a unique secreted protein that interferes with effective host adaptive immunity (Zhong *et al.,* 2001).

2.4. Virulence Genes in Intracellular Bacteria

Plasmids are circular autonomously replicating extrachromosomal deoxyribonucleic acid segment (Abigail and Diexie, 2002). Plasmids may carry virulence genes that can be transferred through conjugation. In addition, plasmids carry insertion sequences or transposons that can further mobilize virulence genes to the chromosome or other plasmids. Plasmids, transposons and integrons may carry antimicrobial resistance genes (Gyles *et al.,* 2004).

Salmonella virulence plasmids (Spy) have been identified in 8.2 kilo base regions of the *Salmonella* genome. The functions of these genes as virulence factors include resistance to the complement mediated bactericidal activity of serum, immune suppressions and intracellular survival and growth (Gulig, 1990). The plasmid is presumed to be important for the interactions of *Salmonella* with the phagocytic cells of reticuloendothelial systems (Krause *et al.,* 1992). The Spy genes expressed by intracellular *Salmonella* have virulence of 10 to 10,000 fold, which varies with the serotype. The plasmid encoded fimbriae (pef gene) which are involved in the attachment of *Salmonella* to the intestinal epithelium and RCK gene which encode for an outer membrane protein is involved in intestinal epithelial cells invasion and resistance to host complement factors (Gyles *et al.,* 2004).

The ability of *Shigella* species to enter epithelial cells and cause disease depends on several unlinked chromosomal genes, as well as virulence genes on a large virulence plasmids termed as invasion plasmids (Sasakawa *et al.,* 1992). These genes encode functions such as attachment to the host cells, induction of endocytosis. intracellular multiplication and spread to adjacent cells (1-lale. 1991; Gyles ci *ul.*. 2004). The products encoded on plasmid for *Yersinia* virulence (pYV) include *Yersinia* adherence (Yad A), attachment invasion low (Au) and antiphagocytic protein called *Yersinia* outer membrane proteins (Yops) (Hirsh and Zee, 1999). These plasmid products enable this pathogen to resist phagocytosis and thus, enhance the survival and growth of *Yersinia* in an intracellular environment (Gyles *et al.,* 2004).

Licithinase, the gene product of *L. monocytogenes* aid in the migration of *Listeria* from cell- to-cell by destructing the double membrane of this microbe. The temperature regulated positive regulatory factor (Prf A) coordinates the expression of several virulence associated genes in *Listeria* (Portnoy *et al.,* 1992).

3. CONCLUSION

Intracellular bacterial pathogens affect a wide range of animal species including human beings. These microbes use different tactics and virulence mechanisms to evade and circumvent the host immunity and thereby ensure their survival and multiplication within host tissues. Understanding the pathogenic mechanisms and the virulence factors of intracellular bacteria are crucial in underlying the pathogenesis and in designing appropriate therapeutic approach.

Hence, research should be conducted at molecular and genetic level to further characterize and understand the pathogenic and virulence factors associated with intracellular bacteria.

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