Exocytosis from gram negative bacteria for *Salmonella* invasion of chicken ileal epithelium

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ABSTRACT

Secretion of membrane-bound vesicles for bulk transport of cellular products for delivery to recipient cells is a well-known phenomenon of eucaryotic cell systems in animals. Such a process of exocytosis has been established for neurotransmission, enzyme secretion and hormone release, wherein a transient or total fusion of secretory vesicular membrane may occur on the cell membrane (Jena, 1997). On the contrary, however, the concept of vesicular exocytosis from procaryotes was unknown till it was discovered recently in *Salmonella* organisms by transmission electron microscopy (YashRoy, 1993) wherein membrane vesicles (MVs) of invading *Salmonella* organisms were seen in intimate contact with the plasma membrane of the host epithelial cells of ileum in *vivo*. This phenomenon has since been confirmed for *Pseudomonas aeruginosa* as well (Kadurugamuwa and Beveridge, 1995); MVs released by these organisms were shown to fuse with outer-membrane of other gram negative organisms in cultures leading to bacteriolysis of the recipient cells. Gram positive organisms also underwent bacteriolysis after coming in intimate contact with these MVs. This discovery of vesicular exocytosis is now providing plausible answers to some pending important questions on the energetic and conformational aspects of secretion of cellular proteins across the outer membrane of gram negative bacteria (Pugsley, 1993), release of cell wall materials (Chatterjee and Das, 1967), initiation of host cell invasion (YashRoy, 1993, 1996) etc., besides being hailed as conceptually new antibiotics against resistant bacteria (Kadurugamuwa and Beveridge, 1995, 1996).

Contains 2 figures

Key words: Gram negative bacteria, exocytosis, membrane vesicles

Cell secretions in gram negative organisms can fill up the periplasmic space between the cell wall and the outer-membrane so as to produce bulbous protrusions from the bacterial surface (Fig. 1) and this process can be augmented upon external stimulation by host cell factors in vivo (YashRoy, 1992) or low concentrations of membrane-destablizing antibiotics like gentamycin in vitro (Kadurugamuwa and Beveridge, 1995). These periplasmic extensions are considered to be filled with toxic secretions produced by the organisms which could help the organisms to invade the host cells (YashRoy, 1993, Ginocchio *et al.*, 1994) and hence it is proposed to name them as 'virulence protrusions'.

It has now become clear that these virulence protrusions represent a stage preceding the release of outer membrane bound vesicles. Their initial naming as 'toxin bombs' (YashRoy,
120

Fig. 1a. Transmission electron micrograph of Salmonella 3, 10:r- organisms (a monophasic variety of otherwise diphasic Salmonella serotypes such as S. weltevreden and S. simii) 18hr after injection into chicken ileum. Most organisms closely interacting with microvilli (mv) of epithelial cells reveal the presence of numerous surface structures (arrows), named as virulence protrusions, arising from the extensions of the periplasm (arrow head) (modified from YashRoy, 1992).

1992, 1993) is more or less vindicated from their composition: autolysins, phospholipase, proteinase, alkaline phosphatase, as established for MVs of Pseudomonas aeruginosa (Kadurugamuwa and Beveridge, 1996). The delivery of these virulence enzymes through an intimate membrane-to-membrane contact with the recipient cell (YashRoy, 1993; Kadurugamuwa and Beveridge, 1996) qualifies this process to be termed exocytosis from gram-negative organisms. This intimate contact was first observed between MVs released from Salmonella organisms and host epithelial cells in chicken ileum in vivo prior to invasion (YashRoy, 1993). Subsequently, employing immuno-electron microscopy, the process has been generalized for MVs released by Pseudomonas aeruginosa fusing with outer membrane of other gram negative organisms besides also attaching to the cell wall of gram positive organisms, leading to their bacteriolysis (Kadurugamuwa and Beveridge, 1996). These membrane vesicles have therefore been projected as, conceptually new antibiotics by Kadurugamuwa and Beveridge (1996).

Proposed mechanism of invasion: Another important role of these exocytotic MVs is connected with the invasion process of gram negative pathogens into host cells. There is evidence to suggest that delivery of the contents of the MVs exocytosed from Salmonella 3, 10:r:- organisms, through intimate contact between bacterial MVs and host cell microvillous membrane, leads to focal disruption of host cell microvilli
Fig. 1b. Transmission electron micrograph of chicken ileum under conditions as above (Fig. 1a) revealing the acquired virulence protrusions (p) and release of membrane vesicles (V) from *Salmonella* 3,10:R:: organisms (S), while closely interacting with microvilli (M) of ileal epithelial cells. The released membrane vesicles have been observed to develop intimate membrane-to-membrane contact (curved arrow) with plasma membrane of host cell microvilli, leading to focal disruption of microvilli and formation of ruffle (empty arrow) in the host cell membrane with the reorganisation of the host epithelial cell cytoskeleton (bold thick arrow) which may facilitate intracytoplasmic entry (invasion) by the pathogen. The *Salmonella* organisms (s) at the site of the ruffle do not show the presence of virulence protrusions (YashRoy, Unpublished).

(YashRoy, 1993) and development of a membrane ruffle by cytoskeletal reorganisation of epithelial cell cytoplasm (Fig. 1b) thereby leading to the intracellular entry of *Salmonella* organisms *in vivo* (YashRoy, 1997). These *in vivo* transmission EM observations (YashRoy, 1992, 1993, 1994 & 1997) are supported by scanning EM observations of Ginocchio *et al.* (1994), wherein shedding of *Salmonella typhimurium* surface appendages preceded ruffle formation in cultured MDCK (host) cells coincident with intracellular entry of *Salmonella* organisms. Based on all these observations a comprehensive mechanism (Fig. 2) for entry of *Salmonella* organisms into the host cells has been recently proposed (YashRoy, 1997). Although, the process of exocytosis in procaryotes (fusion/attachment of MVs with host/recipient cells) was discovered
Fig. 2. A schematic diagram of sequential steps (I, II and III) for entry of *Salmonella* organism into the host epithelial cell (HEC) *in vivo*, based on Fig. 1 and YashRoy (1992, 1993 and 1997). I a: Amorphous structure of cell surface of organisms located away from epithelial cell microvilli representing a non-interactive configuration. 1b: *Salmonella* organisms located around 500 nm off host cell microvilli develop numerous outer membrane-bound periplasmic virulence protrusions (p), filled with a lightly staining material (virulence enzymes). II c: Such activated salmonellae librate outer membrane-bound vesicles (MV) containing virulence enzymes. These membrane vesicles are observed to dock intimately as if to fuse (curved bold arrow) with the host epithelial cell microvillous membrane, thereby discharging vesicular contents to the host cell. This observation marks the discovery of vesicular exocytosis in procaryotes (YashRoy, 1993). III d: The discharge of vesicular contents to the host cells leads to focal disruption of microvilli (curved empty arrows), formation of ruffle (R) in the host cell membrane thus facilitating invasion of the pathogens (d) into the host epithelial cells through cytoskeletal rearrangement (empty straight arrow) thus effected (modified from YashRoy 1997).


**CONCLUSION**

In conclusion, exocytotic membrane vesicles (MVs) released from gram negative bacteria are slated to receive an unprecedented attention of the scientific community: 1) as a novel mechanism of bulk export of cellular proteins without conformational and energetic
constraints of their transport across the bacterial outer-membrane, 2) as an explainable *modus operandi* for the release of membrane bound endotoxin and outer-membrane proteins which eventually may lead to better strategies for handling endotoxic shock in patients, 3) as a method for transfer of DNA from gram negative bacteria to recipient cells, 4) as toxin bombs against host cell defence for invasion of the host cell, which eventually could lead to newer modes of treatment for preventing/combatting gram negative bacterial infections, 5) as new pathogen-free base material for making/improving multiepitope vaccines, 6) as potentially new antibiotics against hard-to-kill gram negative and gram positive infections, etc.

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**REFERENCES**


