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Enteropathogenic *Escherichia coli*-induced alterations in epithelial barrier function

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Διαταραχές της λειτουργίας του επιθηλιακού φραγμού επαγόμενες από την εντεροπαθογόνο *Escherichia coli*

Περίληψη στο τέλος του άρθρου

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Enteropathogenic *Escherichia coli* (EPEC) is a major cause of diarrhea in children throughout the developing world. The diarrhea can be severe, refractory to oral rehydration, protracted, and often lethal. EPEC infection is primarily a disease of children under 2 years of age, although adults exposed to high inocula are also susceptible.[1,2] In the 0–6 month age group, EPEC strains are the most frequently isolated bacterial diarrheal pathogens. In developing countries, 30–40% of infantile diarrhea cases can be attributed to this pathogen.[3–6] EPEC strains are also responsible for sporadic cases of diarrhea in the US and other developed countries. Two studies in Greece identified EPEC in 3.9–5.5% of patients with diarrhea, predominantly children.[7,8] A study in Seattle using diagnostic DNA probes revealed the presence of EPEC-like organisms in 3.6% of the population, a frequency greater than that observed for *Campylobacter* spp, *E. coli* 0157:H7, *Salmonella* spp, *Shigella* spp or *Yersinia* spp.[9] This suggests that the relevance of EPEC as a pathogen in developed countries may be seriously underestimated.

The precise mechanism by which EPEC causes diarrhea is presently not known. Potential mechanisms include the disruption of tight junction (TJ) permeability, alterations in intestinal ion transport and stimulation of intestinal inflammation.[10] This review will focus predominantly on the alteration of TJs by EPEC, and the EPEC factors required for inducing these modifications. Unlike most other enteric bacterial pathogens, EPEC is non-invasive and does not produce toxins. Following attachment, EPEC causes characteristic morphological alterations of epithelial cells known as attaching and effacing (A/E) lesions. These manifest as effacement of microvilli on the intestinal epithelial surface at the sites of bacterial attachment. Actin polymerization below the site of attachment leads to the elevation of the membrane into a pedestal-like structure.[11]

Direct effects on tight junction proteins

EPEC increases transepithelial permeability by directly altering TJ protein phosphorylation and distribution.[10] EPEC also increases permeability by promoting contraction of the perijunctional actomyosin ring. The resulting alteration in barrier function can be measured as a drop in transepithelial electrical resistance (TER). Studies have focused on the effects of EPEC on the transmembrane TJ proteins occludin and claudin-1 and the intracellular TJ-associated protein, zonula occludens 1 (ZO-1). Occludin and the claudin family of proteins have four transmembrane domains that form two extracellular loops in the intercellular space, and participate in the formation of the TJ barrier, possibly by lateral polymerization.[12] ZO-1 is a cytosolic protein that associates with claudins via N-terminal {Note: abbreviation without explanation} (PDZ) domains, and with occludin via a gua-

nylyl kinase domain. ZO-1 also binds to actin, and thus links the TJ to the cytoskeleton. Numerous studies from the laboratory of the authors have demonstrated the modification and movement of these proteins away from the TJ structure following EPEC infection. Infection increases the detergent solubility of ZO-1 and occludin, but not claudin-1.[13] Furthermore, ZO-1 progressively dissociated from claudin-1 and occludin post-EPEC infection. Immunofluorescence confocal microscopy revealed a progressive loss of occludin from TJs and its redistribution along the lateral membrane and into the cytoplasm. Claudin-1 appeared only to migrate down the lateral membrane, without significant re-distribution to the cytosol. Freeze-fracture electron microscopy also revealed claudin-1 containing aberrant strands throughout the lateral membrane of infected cells.

The detailed mechanism by which EPEC alters the distribution of the TJ proteins is presently not known. Dephosphorylation of occludin is known to decrease its association with TJs.[14] It has been demonstrated that EPEC infection of T84 cells results in the progressive dephosphorylation of occluding,[15] corresponding with the removal of this protein from the TJs. Preliminary data suggest that EPEC-induced occludin dephosphorylation may involve a serine/threonine phosphatase since it can be inhibited by calyculin A.

Effects on the perijunctional actomyosin ring

Intracellular calcium levels are likely to be elevated following EPEC infection, constituting one of the signals leading to EPEC-induced alteration of barrier function.[10] The calcium chelator BAPTA-AM abrogated an infection-induced drop in TER without affecting pro-inflammatory responses.[16]

EPEC infection activates myosin light chain kinase (MLCK) in a calcium-dependent manner. The phosphorylation of MLC by MLCK results in the contraction of the perijunctional actomyosin ring thus increasing paracellular permeability. MLCK pharmacological inhibitors and inhibitory peptides could block EPEC-induced MLC phosphorylation, as well as the concomitant decrease in TER.[17,18]

EPEC virulence factors required to alter intestinal permeability

The EPEC factors responsible for altering the TJ barrier and the signaling pathways involved are just beginning to be elucidated. A 35-kb pathogenicity island known as the locus of enterocyte effacement (LEE) was shown to be necessary and sufficient for EPEC infection in *in vitro* studies.[19,20] It was demonstrated that non-pathogenic laboratory strains of *E. coli* harboring a plasmid containing the LEE were able to mimic EPEC for attachment, A/E lesion and pedestal formation.[20] Studies demonstrate that this strain is able to re-distribute occludin and disrupt barrier function.[15]

The five operons in the LEE encode the components of a type III secretion system (TTSS) as well as effector proteins.[19] The TTSS injects effector proteins including the translocated/intimin receptor (Tir) directly into host cells.[21] Upon entry into host cells, Tir is phosphorylated and inserted into host membranes. Interaction of intimin on the bacterial surface with Tir on the host membrane results in intimate attachment to the epithelial cells. Tir phosphorylation initiates the recruitment of various proteins to the site of bacterial attachment and nucleates actin polymerization leading to pedestal formation.[22] Also present in the LEE are the genes for the effector proteins known as *E. coli* secreted proteins (Esp). Of the known secreted proteins of EPEC, EspA, EspB and EspD are required for A/E lesion formation, possibly due to their involvement in delivery of Tir into host cells. Deletion/disruption of the proteins involved in secretion and transport of effector proteins into host cells results in an inability to induce host effects, including TJ alterations.[10]

In contrast to EspA, EspB, and EspD, the effector proteins EspF, EspG, EspH and Map do not appear to be involved in the type III secretion apparatus.[23–25] Thus the *espF* deletion strain, UMD874, secretes wild type levels of EspA, EspB and EspD. Also, UMD874 induces A/E lesions and behaves like wild type EPEC in adherence, ability to induce actin condensation and tyrosine phosphorylation in host cells. However, in sharp contrast to wild type EPEC, UMD874 is attenuated in its ability to perturb epithelial cell TJs.[26] These defects can be complemented by transforming an intact copy of *espF* into the mutant bacteria.

EspF is a proline rich protein with three proline-rich C-terminal repeat sequences. These repeat sequences have been proposed to interact with host proteins and thereby lead to functional consequences. In studies using progressive C-terminal deletions to complement the *espF* deletion strain of EPEC it was determined that the proline-rich repeat sequences were dispensable for inducing barrier function alterations.[27] Current studies are aimed at evaluating the minimum stretch of the N-terminal fragment of EspF required for effecting TJ disruption.

While EspF is clearly involved in disruption of TJs, the contribution of other effector molecules has not been closely examined. In addition, the mechanism by which EspF alters TJs is not known. Possibilities include interactions with and activation of signaling pathways and direct disruption of localization of TJ proteins.

Clearly this is an area of investigation that deserves additional attention.

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ΠΕΡΙΛΗΨΗ

Διαταραχές της λειτουργίας του επιθηλιακού φραγμού επαγόμενες από την εντεροπαθογόνο *Escherichia coli*

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Η εντεροπαθογόνος *Escherichia coli* (EPEC), ένα από τα παθογόνα που προκαλούν διάρροια στα νεογνά, προσκολλάται στα επιθηλιακά κύτταρα του εντέρου, προκαλεί χαρακτηριστικές βλάβες και διαταράσσει τη λειτουργία τους. Ένας μηχανισμός, διά του οποίου η EPEC προκαλεί διάρροια, είναι η διαταραχή της λειτουργίας του επιθηλιακού φραγμού και η αύξηση της διακυτταρικής διαπερατότητας, που επέρχονται ως αποτέλεσμα της ανακατανομής των πρωτεϊνών της στενής επαφής (tight junction), όπως η οκλουδίνη και η κλαουδίνη, καθώς και της συστολής του περί την επαφή δακτυλίου ακτινομυοσίνης. Αν και οι καταρράκτες μεταγωγής των μηνυμάτων, που οδηγούν στην ανακατανομή των πρωτεϊνών της στενής επαφής, παραμένουν ακόμη αδιευκρίνιστοι, έχει δειχθεί ότι η σύσπαση του δακτυλίου της ακτινομυοσίνης είναι αποτέλεσμα ενεργοποίησης της Ca-εξαρτώμενης κινάσης της μυοσίνης. Η παθογενετική δράση των EPEC προϋποθέτει ένα εκκριτικό σύστημα τύπου ΙΙΙ, το οποίο εισάγει δραστικές πρωτεΐνες κατευθείαν μέσα στο κύτταρο. Μια από αυτές τις πρωτεΐνες, η EspF, φαίνεται ότι είναι κρίσιμης σημασίας γι' αυτή τη διαδικασία, επειδή διαθέτει τρεις πλούσιες σε προλίνη επαναλαμβανόμενες αλληλουχίες, που αλληλεπιδρούν με πρωτεΐνες του ξενιστή, και προκαλεί δοσοεξαρτώμενη διαταραχή της λειτουργίας του φραγμού. Παρά ταύτα, ο ακριβής μηχανισμός δράσης της EspF και η μεσολαβητική δράση άλλων εκκριτικών πρωτεϊνών παραμένουν ακόμη αδιευκρίνιστοι.

Λέξεις ευρετηρίου: Εντερικός φραγμός, Εντεροπαθογόνος *E. coli*, Στενή επαφή

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