Effector triggered manipulation of host immune response elicited by different pathotypes of *Escherichia coli*

Elamparithi Jayamani* and Eleftherios Mylonakis

Division of Infectious Diseases; Rhode Island Hospital; Alpert Medical School of Brown University; Providence, RI USA

Keywords: pathogenic *E. coli*, effectors, immunity, type secretion system, virulence, IBD, EPEC, EHEC, AIEC

Effectors are virulence factors that are secreted by bacteria during an infection in order to subvert cellular processes or induce the surveillance system of the host. Pathogenic microorganisms encode effectors, toxins and components of secretion systems that inject the effectors to the host. *Escherichia coli* is part of the innocuous commensal microbial flora of the gastrointestinal tract. However, pathogenic *E. coli* can cause diarrheal and extraintestinal diseases. Pathogenic *E. coli* uses secretion systems to inject an array of effector proteins directly into the host cells. Herein, we discuss the effectors secreted by different pathotypes of *E. coli* and provide an overview of strategies employed by effectors to target the host cellular and subcellular processes as well as their role in triggering host immune response.

### Introduction

In contrast to the adaptive immune system that involves antigen receptors in mediating host surveillance, the innate immune system recognizes pathogens via pattern-recognition receptors (PRR), which triggers a variety of host defense mechanisms. As a result, the innate immune system depends on detecting the molecular structures of pathogens. During an infection, bacterial products or microorganism-associated molecular patterns (MAMPs) stimulate the host immune signaling pathways and defense mechanisms, through several classes of PRR, the best characterized among them are Toll-like receptors (TLR) and nucleotide-binding oligomerization domains proteins (NOD-like receptors, NLRs). Since MAMPs are also present in nonpathogenic or commensal bacteria, the host requires additional strategies to respond selectively against pathogens. Thus, the danger theory was proposed to highlight the damage associated microbial proteins (DAMPs) via PRRs, which alerts the host to respond to the pathogens by triggering the immune response. The host immune system responds to the indirect evidence of the pathogen by sensing disruption of cellular processes caused by a battery of microbial effectors secreted by various pathogens in a phenomenon termed as effector-triggered immunity (ETI). The ETI is conserved across several organisms ranging from invertebrates *Caenorhabditis elegans* and *Drosophila melanogaster*, mammals and across the plant kingdom. The adaptive immune system comprising T cells and B cells, recognizes these effectors as pathogenic antigens and equips the host with long-lasting immunological memory. Effector proteins trigger cytokine release and, acting in concert with PRR activation by MAMPs and DAMPs, activate T and B cells.

Bacteria use the secretion system to sense their environment within the host. Type secretory systems are molecular machines that evolved among gram-negative bacteria, which translocate or export effector proteins across both outer and inner membranes and also inject the effectors into a host. Thus, the bacterial effector proteins are products of co-evolution with host. The effectors secreted by bacteria play significant role in subverting host cellular processes or in effector triggered immunity. Thus effectors are very important for the pathogen to establish the infection in the host. Most of the effector proteins are secreted and introduced into host cells by type III (T3SS), type IV (T4SS), and type VI (T6SS) secretory systems. Bacterial toxins are secreted by type I, type II, and type V secretory system. The type VII (T7SS), also known as the early secretory antigenic target 6 (ESAT-6) systems 1 (ESX-1) pathway, and type VIII (T8SS) secretory systems have also been reported recently.

*E. coli* is the most abundant facultative anaerobe of the microbiota of the human gut, and can normally be found colonizing the mucus layer of the colon. Recently, a number of pathogenic strains of *E. coli* have adapted to other niches, causing diverse intestinal and extraintestinal diseases. The emerging new traits of *E. coli* implicated in intestinal diseases are due to loss and gain of genetic elements through horizontal gene transfer (HGT) which enables the bacteria to become highly diverse and better adapted to their various niche. The acquisition of the pathogenicity islands through HGT equips the bacteria to attain the pathogenic traits for their survival in the host. The pathotypes of *E. coli* causing enteric disease are enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC), which use their secreted proteins or effector repertoire to target the host signaling pathways. EPEC and EHEC form attaching and

*Correspondence to: Elamparithi Jayamani; Email: elamparithi_jayamani@brown.edu

Submitted: 04/28/2014; Revised: 07/08/2014; Accepted: 07/15/2014; Published Online: 08/04/2014; http://dx.doi.org/10.4161/viru.29948
This review will highlight some selected examples and isolated from the ileal mucosa of a patient with Crohn disease unexplored adherent-invasive (AIEC) pathotype, which was first cells by using fimbriae or pili for attachment, and the relatively invasive (EIEC; which, unlike other pathotypes, adheres to host damage and tight junction disruption. Other E. coli disease, perturbations in nutrient/water uptake system, microvilli or EHEC infection causes mitochondrial dysfunction, diarrheal these two pathogens are also known as A/E pathogens. EPEC causing distinct pedestals beneath the site of attachment, thus effectors and toxins of E. coli EHEC shares many virulence strategies with EPEC despite having distinct clinical manifestations. EPEC has a plethora of type III secreted effectors that manipulate host cellular processes to enable their replication inside the cells. Therefore, enteropathogenic E. coli effectors play a vital role in the evolutionary competition between the pathogen and the host. The EPEC genome contains at least seven effector genes in the region of locus of enterocyte effacement (LEE) (Tir, Map, EspF, EspG, EspZ, EspH, and EspB) and few non-LEE (Nle)-encoded effector genes (Table 1). These effectors use T3SS for delivery into host cells. Although T2SS is also known to exist in EPEC, the substrates and role in virulence are scarcely reported. EPEC also secretes enterotoxin EspC and putative transporters that have been reported to act through T3SS even though they are usually substrates for type five-secretion system (T5SS) (Table 1).

| Table 1. EPEC effectors and their physiological functions |
|----------------------------------------------------------|
| EPEC effector | Physiological functions | References |
|-----------------------------------|-----------------------|------------|
| Tir | Cell detachment | 43 |
| Map | Filopodia formation | 89 |
| | Mitochondrial dysfunction | |
| | Microvilli effacement | |
| EspB | Anti-phagocytosis | 55 |
| | Microvilli effacement | |
| | Actin disruption | |
| EspF | Microvilli effacement | 83 and 84 |
| | N-WASP activation | |
| EspH | Modulating actin dynamics | 50 and 53 |
| | Cytoskeleton disruption | |
| EspZ | Tir, EspF, and Map regulation | 88 |
| EspG | Microtubule disruption | 59 |
| | TJ disruption | |
| | Paracellular permeability | |
| | Aquaporin redistribution | |
| | Stress fibers formation | |
| | DRA transporter inhibition | |
| NleH1 | Pro-inflammatory | 67 |
| NleB2 | Apoptosis inhibition | 70 |
| NleC | NF-kB inhibition | 72 |
| NleD | c-Jun N-terminal kinase (Jnk) inhibition | 38 |
| NleG | Act as E3 ubiquitin ligase mimic | 75 |
| NleH2 | Pro-inflammatory | 67 |
| NleA | Inhibition of protein secretion by interference with COPII | 62 |

Effacing (A/E) lesions on the intestinal epithelial cells, thereby causing distinct pedestals beneath the site of attachment, thus these two pathogens are also known as A/E pathogens. EPEC or EHEC infection causes mitochondrial dysfunction, diarrheal disease, perturbations in nutrient/water uptake system, microvilli damage and tight junction disruption. Other E. coli pathotypes include enterotoxigenic (ETEC), enteropathogenic (EPEC), uropathogenic (UPEC), diffusely adherent (DAEC), enteroinvasive (EIEC; which, unlike other pathotypes, adheres to host cells by using fimbiae or pili for attachment), and the relatively unexplored adherent-invasive (AIEC) pathotype, which was first isolated from the ileal mucosa of a patient with Crohn disease (CD). This review will highlight some selected examples and strategies involving the pathogenic effectors and toxins of E. coli that trigger the immune responses or subvert host cellular processes while establishing the infection.

**Pathotypes of E. coli**

**Enterohemorrhagic E. coli** (EHEC)

EHEC is a highly infectious A/E pathogen that causes bloody diarrhea and hemolytic uremic syndrome (HUS) throughout the world. A major virulence strategy employed by EHEC is production of Shiga toxin (Stx). EHEC contains genomic island LEE which encodes T3SS and its substrates like EspA, EspB, NleH1, NleH2, and Tir. EHEC shares many virulence strategies with EPEC despite having distinct clinical manifestations. Enteropathogenic E. coli (EPEC)

EPEC has a plethora of type III secreted effectors that manipulate host cellular processes to enable their replication inside the cells. Therefore, enteropathogenic E. coli effectors play a vital role in the evolutionary competition between the pathogen and the host. The EPEC genome contains at least seven effector genes in the region of locus of enterocyte effacement (LEE) (Tir, Map, EspF, EspG, EspZ, EspH, and EspB) and few non-LEE (Nle)-encoded effector genes (Table 1). These effectors use T3SS for delivery into host cells. Although T2SS is also known to exist in EPEC, the substrates and role in virulence are scarcely reported. EPEC also secretes enterotoxin EspC and putative transporters that have been reported to act through T3SS even though they are usually substrates for type five-secretion system (T5SS) (Table 1).

**Adherent-invasive E. coli** (AIEC)

Adherent-invasive E. coli (AIEC) are observed in the ilea of 36.4% of patients with Crohn disease. Abnormal expression of carcinoembryonic antigen-related cell adhesion molecule (CEACAM) in the gut of Crohn disease patients facilitates the adherence of AIEC via type I pili expression. Interestingly, CEACAM expression is triggered by adherence of AIEC through the stimulation of tumor necrosis factor (TNF) and gamma interferon, leading to an inflammatory condition.

The ability of AIEC to replicate inside the macrophage without inducing programmed cell death accelerates TNF-α release in the host. TNF-α is a key cytokine involved in inflammation, that has been implicated in IBD. Intestinal dysbiosis is a prerequisite as well as cause for IBD. The high prevalence of AIEC and its persistent infection in the gut of IBD patients could be due its repertoire of virulence factors and effectors secreted in the gut. The genome sequence of AIEC shows the presence of genes encoding T6SS along with the fimH adhesion protein, long polar fimbiae, and other virulence factors. The genes encoding various components of T6SS are involved in the context of translocation of effectors into both prokaryotic and eukaryotic cells. AIEC containing this special apparatus to inject the toxins to other bacteria may have an edge over other commensals present in the gut. Further studies on AIEC virulence mechanisms associated with T6SS will give more insight into AIEC secreted proteins and their role in gut dysbiosis and its contribution to inflammatory bowel diseases.

**Uropathogenic E. coli** (UPEC)

The E. coli pathotype encodes several virulence genes that aid in colonizing the urinary tract and establishing persistent infection in face of effective host defense. The effector cytotoxic necrotizing factor 1 (CNF-1) and hemolysin (HlyA1) are toxins produced by uropathogenic E. coli (UPEC). It has been reported that the effector CNF-1 is internalized via receptor-mediated endocytosis and modified the host protein, the RHO-family GTPase Rac2. The effector CNF-1 activates Rho-GTPase in deamidation of glutamine 63 of RhoA and glutamine 61 in Rac2 and Cdc42. Consequently it leads to cytoskeletal alterations, multinucleation with cellular enlargement and finally induces...
the transcription factor Nck-B that increases the host pro-survival strategy. The modified form of Rac 2 interacts with immune adaptor protein IMD leading to the activation of immune signaling pathways. Importantly, the immune response by the host is not triggered by direct recognition of the virulence factors but in response to the activation and modification of the host proteins by the effector. Thus, the ETI response to such effectors confirms indirect way of sensing the infection is widely conserved in plants and animals.2,9,21

Manipulation of Host Cellular and Subcellular Targets by E. coli effectors

Bacterial effectors subvert host cellular processes in multiple ways, such as targeting kinase-signaling cascades, manipulating the cell death pathways, modifying host cell membranes and subsequently targeting multiple organelles, targeting ubiquitin, a posttranscriptional modifier which plays critical role in several cellular processes (Fig. 1).38-41

Manipulation of host cytoskeleton by the effectors

The success of a pathogen depends on its ability to infiltrate the host tissues by attaching to cells. Effectors target host pathways that control important cellular functions like actin and cytoskeletal dynamics facilitate the adhesion and colonization of bacteria.23 The EPEC and EHEC effector Tir is translocated to the host cell cytoplasm by T3SS and targets actin polymerization.28 Subsequently, Tir associates with the host membrane where it acts as a receptor for intimin and on interaction with intimin, Tir form clusters.42 The host tyrosine kinases phosphorylate the clustered Tir which leads to the recruitment of the adaptor protein Nck2 to site of interaction.43 The Tir-intimin-Nck2 complex activates the Wiskott Aldrich Syndrome protein (WASP) to promote actin polymerization.43 WASP and N-WASP are actin nucleation promoting factors (NPFs) with well-characterized roles in promoting actin polymerization.44,45 Mutations in NPFs affect the stability of the protein as well as the decrease in binding efficiency with key interacting partners, thereby causing the genetic disease Wiskott Aldrich Syndrome, an immunodeficiency disorder.66,67 NPFs promote actin polymerization by activating the Arp 2/3 complex which is made up of seven subunits including Arp2 and Arp3.48 WASP and the Arp 2/3 complex proteins are conserved across eukaryotic organisms ranging from unicellular yeast to metazoans and regulate cellular processes dependent on the actin cytoskeleton.44,46-49 Thus, Tir effectors subvert the host cytoskeleton in order to enable the bacteria to adhere to the host cell through actin pedestals and additionally cause microvilli effacement.45

EspH is another T3SS effector protein, which in addition to Tir, is involved in actin dynamics by playing a major role in pedestal elongation and formation.50 EspH differs from Tir in that it interacts with Nck and IRSp53/ITRKS. IRSp53 has been shown to bind with N-WASP and is involved in causing eukaryotic cell membrane projection and fusion that is mediated through actin cytoskeleton.51,52 EspH has been shown to promote actin polymerization and this function is inhibited by a dominant negative isoform of WASP-interacting protein (WIP).53 WIP binds to N-WASP WH1 domain to inhibit the EspH activity in actin dynamics. On the other hand, EspH recruits N-WASP and Arp2/3 to the site of bacterial attachment. The C-terminus region of Tir and WH1 domain of WASP mediate this process. Therefore, the involvement of EspH in actin polymerization is independent of but also synergistic with signaling cascades activated by Tir.28 Thus, the influence of one effector on the activity of another effector is very significant in the context of persistent bacterial infection in the gut.

Recent studies by Yosuke et al. showed that EspB inhibits phagocytosis by binding to myosins that interact with actin filaments in mediating cellular processes.54 EspB needs to interact with another effector EspA for its translocation. Several plasmids encoding various regions of EspB showed different functional characteristics.55-56 The EspB effector has multiple roles during EHEC infection, including pore formation, adherence and translocation of other effectors. It is also known that EspB secreted by EHEC interacts with α-catenin at the infection site.57

The alteration of cytoskeleton dynamics upon EPEC infection mediated by EspG was observed.58 EspG binds with the golgi matrix protein GM130 leading to the disruption of golgi apparatus of the host by affecting vesicular trafficking, which leads to the inhibition of the normal ARF GTP/GDP cycle that
is vital for membrane transport events. In addition to ARF inhibition, EspG activates PAK kinase activity by binding adjacent to the ARF binding site. The unique complex formation by EspG with eukaryotic partners ARF and PAK was shown to be involved in manipulation of membrane traffic as well as affecting the cytoskeleton.

The effector NleA targets the host cellular dynamics, in addition to their contribution to the EPEC pathogenicity. Kim et al., showed that NleA interacts and inhibits mammalian coat protein complex II (COPII) protein. COPII is necessary for intracellular protein transport for binding with Sec24. Deletion studies showed that the highly conserved C-terminal ETRV motif in NleA contributes to the perturbation of COPII protein dynamics. Moreover, overexpression of NleA affects the cellular secretion and protein export in ER. Recently NleA has been implicated in intestinal tight junction disruption upon infection. It has been hypothesized that NleA may be affecting tight junction repair pathway by blocking the cargo transport of new proteins. Thus, various effectors independently as well as synergistically contribute to microvilli effacement, actin and cytoskeletal dynamics.

**Effectors targeting host-signaling cascades**

In general, host cells respond to EPEC and EHEC infection by increasing NF-κB p50/p65 heterodimer binding affinity to DNA and initiate the expression of target genes involved in defense as well as cytokine production. Ribosomal protein S3 (RPS3) is a binding partner of NFκB that facilitates DNA binding. However, type III secretory system effectors NleH1 and NleH2 are able to bind with RPS3. Thus, NleH1 prevents IKKb from phosphorylating RPS3, eventually inhibiting RPS3 translocation into the nucleus, which halts the upregulation of RPS3 dependent genes. The direct binding of NleH to NF-κB subunit and its ability to manipulate the major regulator of innate and adaptive responses reveal its significance.

NleH effector has been reported to affect the process of programmed cell death (PCD), the genetically controlled or regulated forms of death that can be categorized into apoptosis, necrosis, and pyroptosis. During infection, immune defenses are triggered by the activation of inflammatory pathways, complement system, and antimicrobial peptides production at the site of infection. Moreover, the unresolved condition of infection leads the activation of PCD in order to eliminate the intracellular proteins or effectors inside the cells. Either induction or suppression of PCD can help pathogens to evade various host defenses or to replicate and proliferate inside the cells respectively. During infection, NleH effectors inhibit the increase in Ca²⁺ accumulation in the cytoplasm, caspase-3 activation and nuclear condensation to promote cell survival. Cytoprotective BI-1, a trans-membrane protein associated with ER membrane has cytoplasmic facing N-terminal with about 40 amino acids. Interestingly, NleH effectors inhibit apoptosis by exploiting the binding site of BI-1 extended into the cytoplasm. Cell death signaling pathways are the last resort of innate immune responses in order to eliminate the infected cells by process of engulfing. Subsequently, engulfed cells are presented to T cells in order to induce the adaptive immune system. Moreover, bacterial effectors are evolved strategies to overcome the host responses by inhibiting apoptosis of the host cell.

Recently, the effector NleB was shown to be the first known virulence factor targeting death receptor signaling. Two independent reports showed that NleB binds with cell death-domain protein, which in turn leads to the inhibition of the death receptor mediated pathway. NleB inhibits Fas ligand binding leading to TNF induced formation of canonical death-inducing signaling complex. The N-acetylglucosamine transferase activity of NleB inhibits Arg117 and Arg235 residue in FADD (Fas associated death domain) and TRADD respectively. Studies in HeLa cells infected with EPEC and EPEC NleB mutant at a catalytic site showed profound effects on apoptosis. Thus, NleB down-regulates the caspase-8 activation and mediates the inhibition of apoptosis.

NFκB and c-Jun N-terminal kinase (Jnk) are the two major pathways contributing to inflammatory response upon infection. The effector NleC downregulates the induction of inflammatory response IL-8 mediated through NFκB inhibition. The NleC, Zn-dependent metalloproteases cleaves RelA (p65), a subunit of NFκB leading to transcriptional inhibition of immune response genes. Also, manipulation of the host-kinase signaling cascade by NleC is observed, where it inhibits phosphorylation of p38 MAPK (mitogen activated protein kinase). NleD, the second Zn endopeptidase effector, specifically targets JNK by affecting one of the major arm of inflammatory response. It specifically inactivates JNK and p38 kinase by acting on their activation loop. Thus, NleC and NleD inhibit NFκB/p38MAPK and JNK/p38 MAPK, respectively. They act in co-operation as well as in complement each other to downregulate IL-8 production.

The distinct nature of NleG resemblance to the host U-box/RING E3 ubiquitin ligases has gained enormous importance. Ubiquitin is a highly conserved polypeptide with 76 amino acids with the property of reversible posttranslational modification. Ubiquitination of proteins occurs in host cells in order to sort the proteins for degradation as well as for other cellular processes. Usually, the 26S proteasome machinery carries out ubiquitination of proteins. Three classes of enzymes regulate this process, namely: ubiquitin activating enzyme (E1), ubiquitin conjugating enzyme (E2), and ubiquitin-protein ligase (E3). The E3 ligase is further divided into two categories based on their functional motif, HECT (homologous to E6-associated protein C-terminus) domain and RING (really interesting new gene) domain (a modified RING motif). Ubiquitin proteasome system (UPS), with an extensive usage in host cellular processes, is a natural target of bacterial effector proteins. The presence of E3 ligase mimic in the C-terminal region of NleG allows them to interact with E2 enzymes in a similar manner to the normal E2/E3 ligase interaction of the host. The increasing interest on the role of ubiquitin in regulating various signaling cascades in eukaryotes will enable a better understanding of the exploitation of host ubiquitilation system by prokaryotic effectors.

**Effectors targeting to mitochondria**

In eukaryotic cells, pre-proteins are recognized and translocated to the mitochondria in an unfolded state by transporter outer domain (TOM) and transport inner domain (TIM) membrane
complexes present in the mitochondria. The effectors Map and EspF are secreted into the host cell by T3SS. Both of them contain a putative N-terminal mitochondrial pre-sequence with cleavage signals, which directs its localization to the mitochondria. At the mitochondria, the pre-sequence is cleaved, subsequently transported by mitochondrial importing machineries TOM22, TOM40 and mitochondrial chaperon HSP70, along with EPEC effector Map and EspF localization.

Increasing numbers of bacterial toxins and effectors have been reported to target mitochondrial function, where they cause mitochondrial dysfunction and regulate apoptosis. Recently it has been shown in C. elegans that the mitochondrial dysfunction by microbial effectors induces the surveillance pathway such as mitochondrial repair, upregulation of detoxification and innate immune pathway.

EspF is known for its multifunctional role when compared with other effector proteins. EspF consists of three proline-rich repeats (PRR) and six domains of putative Src homology 3 (SH3). It acts along with N-WASP in remodeling host actin cytoskeleton, indicating its involvement in manipulating several eukaryotic signaling pathways. EspF was also found to cause nuclear factor mobilization, which in turn affects ribosomal processing, leading to translational inhibition. The evidences of translational inhibition by bacterial toxins and effectors leading to effector-triggered immunity were elucidated in C. elegans, which has emerged as a popular model for studying host-pathogen relationship and anti-infective drug discovery. In C. elegans, the induction of the transcription factor zip-2 was observed upon translational inhibition by bacterial toxin and zip-2 induces the innate immune genes to protect the worms from bacterial toxins. This phenomenon of indirect sensing of pathogen attack in the host and ETI response may be well conserved in human defense system in order to differentiate innocuous microbes from pathogen.

The interesting aspect of EspZ is its role in regulating the translocation of other T3SS effectors from EPEC and EHEC. EspZ was characterized as a pro-survival mediator that can prevent apoptosis and the protein was shown to localize to mitochondria. Mutation in EspZ affects secretion of these T3SS effectors in the cytoplasm. Ectopic expression of EspZ leads to abrogation of actin pedestal formation. This inhibition is due to EspZ control over the translocation of other effectors like Tir, EspF, and Map. Thus, EspZ may act as a host-protective virulence factor.

**Conclusion**

In the past decade, significant progress has been made in understanding different effector proteins and toxins secreted by several E. coli pathotypes. These effectors are able to manipulate or subvert various host cellular processes such as kinase signaling cascades, the ubiquitin proteasome system, actin cytoskeleton dynamics and programmed cell death. The effectors also act as host-protective virulence factors by preventing host cell lysis. Studies on E. coli pathotypes and their effector proteins is intriguing, not only because of their central role in enteric diseases like diarrhea, but also for their role in gut dysbiosis. The availability of genome sequences of various new pathogenic E. coli will provide more information on host-pathogen co-evolution in the context of effector-triggered immunity in the gut.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

The authors wish to thank Jonah Larkins-Ford and Dr Ralph Rogers for their critical reading of the manuscript.

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