Published in final edited form as:

Curr Opin Infect Dis. 2010 October ; 23(5): 464–469. doi:10.1097/QCO.0b013e32833dcebd.

An inside job: subversion of the host secretory pathway by intestinal pathogens

Tyler M. Sharp and Mary K. Estes

Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas, USA

Abstract

Purpose of review—The cellular secretory pathway, composed of the endoplasmic reticulum, Golgi apparatus, and cellular vesicles, mediates the intracellular trafficking of proteins and lipids. Gastrointestinal pathogens frequently affect the functions of enterocytes, the differentiated cells involved in secretion and absorption of extracellular molecules. Microbial pathogenesis can be enhanced by altering the trafficking of key molecules such as brush border enzymes, soluble immune mediators such as cytokines and chemokines, and MHC Class I molecules, all of which rely on the secretory pathway for their appropriate cellular localization. This review focuses on our current understanding of the distinct mechanisms employed by enteric pathogens to antagonize the secretory pathway.

Recent findings—Many pathogens encode individual or multiple proteins to antagonize the secretory pathway, including disrupting the trafficking of vesicles between the endoplasmic reticulum, Golgi, and plasma membrane. This antagonism allows for increased pathogenesis and can assist, directly or indirectly, in microbial replication. Virtually all arms of the secretory pathway are targeted by intestinal pathogens, supporting the pathogenic significance of shutting this pathway down.

Summary—This review summarizes the mechanisms utilized by gut pathogens to disrupt the cellular secretory pathway and addresses potential therapeutic targets to combat these highly prevalent and burdensome microbes.

Keywords

E. coli; enterovirus; norovirus; rotavirus; secretory pathway

Introduction

Diarrhea is the cause of more than two million deaths worldwide annually [1], and in the USA alone there are 211–375 million cases that lead to approximately 900 000 hospitalizations and 6000 deaths [2,3]. Infectious agents are the cause of nearly all diarrheal morbidity and mortality, and elucidation of the mechanisms used by microbes to disrupt

critical host pathways can facilitate the development of effective measures to combat these pathogens.

The secretory pathway is responsible for processing and sorting proteins, for biogenesis and metabolism of lipids, and for trafficking these proteins and lipids to various subcellular compartments, including those destined for secretion out of the cell. As such, the secretory pathway is increasingly being implicated in contributing both directly and indirectly to a wide variety of disorders [4,5], including diarrheal diseases related to abnormalities in ion transport (reviewed in [6]).

Much knowledge of the function of the secretory pathway has been gained by the use of secretory pathway antagonists, primarily of microbial origin. Brefeldin A (BFA), a potent antagonist of trafficking between the endoplasmic reticulum (ER) and Golgi, has been used extensively to study the dynamics of Golgi assembly and protein trafficking [7], and Shiga toxin revealed for the first time nonclassical retrograde transport to the ER [8,9]. Thus, there can be great benefit to studying microbial proteins that interact with the secretory pathway not only for therapeutic benefit, but also as tools to understand molecular processes that regulate cellular protein trafficking and secretion.

A variety of microbes exploit the secretory pathway for their replication [10–12], and a growing body of evidence implicates the secretory pathway as a specific site of attack by intracellular pathogens to enhance virulence and/or antagonize host-cell function (Table 1) [13,14•, 15•,16–26] (Sharp *et al.*, in preparation). Here we briefly summarize recent work on intestinal pathogens with particular emphasis on common points of attack and the downstream consequences of antagonism of this critical cellular pathway.

Escherichia coli

Escherichia coli is the leading cause of travelers' diarrhea [27,28], causing approximately 400 million diarrheal episodes and 380 000 deaths in children less than 5 years of age every year in the developing world [28,29]. Although *E. coli* replicates outside of cells, the combination of a type III secretion system that injects bacterial proteins into cells and a toxin that alters protein sorting has made some *E. coli* strains efficient inhibitors of protein secretion. NleA [non-LEE (locus for enterocyte effacement pathogenicity island)-encoded effector A] is a virulence factor whose presence correlates with severe enterohemorrhagic *E. coli* O157:H7 infections [30,31] and that is absent from nonpathogenic *E. coli* [32–34]. NleA directly interacts with Sec24, a component of COPII vesicles, to compromise the trafficking of these vesicles and, therefore, inhibit cellular protein secretion [13].

NleA also functions with the *E. coli* proteins EspF and Map in disruption of intestinal tight junctions during infection, a role that can be mimicked by treatment of cells with BFA following infection with NleA-deficient *E. coli* [14•]. This suggests that antagonism of the secretory pathway is necessary for further downstream effects of various secreted *E. coli* proteins and demonstrates the significance of alterations in protein secretion not only on the host cell directly but also on the pathogenic function of additional microbial proteins.

Many strains of *E. coli* also produce Shiga toxins, well known inhibitors of protein synthesis [35,36] whose presence is associated with severe disease [37,38]. The mechanisms by which Shiga toxins enter and traffic within cells has been elucidated [39]; however, their role in induction of diarrhea remains largely unknown. A recent study of Shiga toxin 1 (Stx-1) demonstrated that it increases galectin-3 secretion from enterocytes *in vitro* and *in vivo* resulting in altered trafficking of various brush border proteins [15•]. This suggests a direct role for Stx-1 in the induction of diarrhea in *E. coli* cases, as these enzymes are critical for the adsorptive function of enterocytes.

Several bacteriophages transfer Shiga toxins to *E. coli*, thereby driving susceptible strains towards pathogenesis [40,41]. Interestingly, an NleA-like type III effector protein has been found in an Stx-1-converting bacteriophage [42], demonstrating that individual bacteriophages are capable of transferring both proteins characterized as antagonists of protein secretion to susceptible strains of *E. coli*. Specific elucidation of the independent and concerted effects of NleA and Stx-1 on the secretory pathway will shed much light on the significance of this pathway in *E. coli* pathogenesis.

Picornaviruses

The picornaviruses are well established as models for many positive strand RNA viruses that replicate in the cytoplasm of infected cells and whose genome functions as a messenger RNA. Expression of viral proteins made from a large self-cleaving polyprotein induces significant rearrangements of cellular membranes that are required for genome replication, and early studies asked if such rearrangements of cellular membranes affect the efficiency of the secretory pathway. After the observation that independent expression of two poliovirus (PV) proteins, 2B and 3A, induced membrane rearrangements similar to those observed during PV replication, it was found that these two proteins also inhibit the secretion of both endogenous and reporter proteins [43]. Further efforts demonstrated that 3A blocks protein secretion by specifically inhibiting ER/Golgi trafficking [44,45], resulting in a large decrease in expression of MHC class I molecules [46] and the tumor necrosis factor receptor [47] from the cell surface. These effects coupled with reduced release of cytokines that normally alert immune cells and initiate antiviral defenses [48] ultimately result in a broad inhibition by 3A of both the induction of apoptosis and clearance of virus-infected cells by lymphocytes. Although this antagonism does not significantly affect the ability of the virus to replicate, it does lead to a decrease in the immune response to infection, thereby allowing for a prolonged period of viral replication before clearance of infected cells and a concomitant increase in viral pathogenesis [20,44,48].

Interestingly, antagonism of protein trafficking by 3A is not conserved in all picornaviruses, but only exists in the enteroviruses PV and coxsackie virus B3 (CVB3) [21,49], which have at least part of their life cycle in the gastrointestinal tract. Molecular analysis of the CVB3 3A protein demonstrated that it binds and debilitates GBF1, a guanine-exchange factor necessary for the activation of Arf1, a critical component of COPI vesicle biogenesis [20]. This activity is engendered by the N terminus of the protein [19,50] that is conserved in PV, but not other picornaviruses [19], mirroring the observed effects of picornavirus infection on the secretory pathway.

Although the 2B proteins from PV and CVB3 also antagonize the secretory pathway [21,43], little is currently known about their mechanism of action. However, 2B has been characterized as a viroporin, in that it forms pores in intracellular membranes allowing the passage of ions and small molecules [22–24,51]. The prominent and critical role of the secretory pathway in the storage and release of calcium ions [52,53] raises the possibility of secretory pathway antagonism being a direct or indirect effect of viroporin formation by 2B. The secretory pathway's critical role in maintaining cellular ion homeostasis raises the possibility that the 2B viroporin either directly or indirectly antagonizes this pathway.

Rotaviruses

Rotaviruses are the leading cause of life-threatening diarrheal disease among infants and young children and results in approximately 600 000 annual deaths worldwide [54]. An unusual feature of rotavirus replication is that while the virus produces two glycoproteins, neither of these traffic to the Golgi, and rotavirus particles and nonstructural glycoprotein 4 (NSP4) both are released into the media of infected polarized cells by a nonclassical trafficking pathway [55,56]. Early studies demonstrated the mistrafficking of apical brush border hydrolases during rotaviruses infection [57,58], and infection has recently been associated with a deficiency in an inflammatory cytokine response expected for a viral infection of the gastrointestinal tract [59]. Together, these observations suggest a specific antagonism of the secretory pathway, resulting in a downregulation of the cellular cytokine response to rotavirus infection. NSP4 has pleiotropic roles in rotavirus replication and pathogenesis [60]; most prominently, it is the first described viral enterotoxin, inducing diarrhea upon independent administration to susceptible animals [61]. One proposed mechanism of pathogenesis by NSP4 is inhibition of protein trafficking within the early secretory pathway, which the protein carries out by binding to microtubules [18]. Though not yet well elucidated mechanistically, NSP4 may be individually responsible for alterations in the cytokine response to rotavirus infection, reinforcing its importance as an antiviral drug target to combat severe disease [62].

Noroviruses

Noroviruses (NoV) affect individuals of all age groups and are a major cause of sporadic cases and epidemic outbreaks of gastroenteritis worldwide [63,64]; they cause approximately 23 million cases of diarrhea annually in the USA alone [2] and approximately 200 000 deaths in children under 5 years of age in the developing world [65]. Similar to the enteroviruses, NoV encode two proteins whose independent expression leads to alterations in protein trafficking. The NoV N terminal protein (NTP) induces significant rearrangement of the Golgi [16] and inhibits surface expression of VSV G by antagonizing an intra-Golgi or post-Golgi step in protein trafficking [17]. These alterations are likely mediated by interaction with the cellular protein VAP-A, a critical regulator of SNARE-mediated vesicle fusion in the secretory pathway [17]. Homology of NTP with PV 2B in both sequence and function suggests that NTP may have additional functions as a viroporin, although these studies have yet to be carried out.

Recent studies on the Norwalk virus nonstructural protein p22 revealed that it is also an inhibitor of protein secretion. Similar to NleA, p22 antagonizes the secretory pathway by affecting the proper trafficking of cellular COPII vesicles; however, p22 accomplishes this with dependence on a mimic of a cellular ER export signal (MERES), which are signals used to promote the uptake of cellular proteins into COPII vesicles to expedite their transport to the Golgi (Sharp *et al.*, in preparation). Thus, p22 mimics a cellular motif to gain access to the secretory pathway and ultimately inhibit it. This has proposed the p22 MERES motif as a site for antiviral drug design as it is well conserved in NoV homologues of p22 but distinct from cellular signals.

Therapeutic targets

The paramount roles of the secretory pathway in maintaining cellular homeostasis and in trafficking lipids and proteins, including modulators of the immune response, has made this pathway a prime target for antagonism by microbial proteins (Fig. 1). These antagonists are, therefore, ideal candidates for antimicrobial drug design. Inhibition of these antagonists may reduce the pathogenesis of the infecting organism. For example, abrogating secretory pathway antagonism by PV 3A or infection with enteropathogenic *E. coli* containing an attenuated NleA diminishes *in vivo* pathogenesis [13,20,43,46]. These and other possible therapeutic targets should be explored in greater detail, as their function in pathogenesis seems clear, although the possibilities and consequences of their inhibition remain largely unexplored.

Conclusion

Study of antagonism of the secretory pathway by enteric pathogens remains in its infancy; however, studies on a variety of microbes that infect the gastrointestinal tract have demonstrated the importance of attacking this pathway during microbial infection. Nevertheless, much work remains to be carried out and many questions have yet to be clearly answered. The function of enterocytes in continually secreting and absorbing various micro- and macromolecules from the intestinal milieu makes them an attractive model to study the secretory pathway and how select pathogens counteract it. Specific attention should be placed on less studied intestinal pathogens that may benefit from such antagonism, for example *Campylobacter* [66], as well as adenovirus-40 (AdV-40) and -41, etiologic agents of diarrhea mostly in infants [67] that also account for approximately 4% of all cases of diarrhea in immunocompetent individuals [68]. Interestingly, recent reports have shown that the protein product of E4orf4 from the respiratory pathogen adenovirus-2 (AdV-2) inhibits protein secretion by targeting recycling endosomes to the *trans* Golgi, resulting in dispersion of the Golgi and ultimate cell death [25,26]. This suggests conservation of function in other AdV, namely Ad-40 and Ad-41, as E4orf4 is well conserved between these strains.

In addition to providing new therapeutic targets, dissecting the molecular mechanisms employed by secretory pathway antagonists will also enhance our understanding of the cell biology of this pathway so elegantly exploited by these deadly and costly pathogens.

Acknowledgments

We thank Sue Crawford, Joseph Hyser, David Graham, Rick Sifers and Wayne Lencer for insightful discussion. Work in the authors' laboratory is funded by the Public Health Service grants from the National Institute of Health P01 AI057788, RO1 AI080656 and P30 DK 56338 (MKE), T32 AI04741 (TMS), and by the East Asia and Pacific Summer Institute grant 7316 (TMS) from the National Science Foundation.

References and recommended reading

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- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 535).

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demonstrated that NleA, in combination with other *E. coli* proteins, affects tight junction formation by altering the trafficking of proteins critical to their formation

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Figure 1. Inhibition of the cellular secretory pathway by intestinal pathogens

The cellular secretory pathway begins at the endoplasmic reticulum (ER) where COPII vesicles package cargo, bud from ER exit sites, and traffic along microtubules to the ER/Golgi intermediate compartment (ERGIC). COPI vesicles form at the ERGIC and traffic cargo to the Golgi. Secretory vesicles form at the Golgi to traffic cargo destined for cell surface expression or for secretion out of the cell. Endosomes take up and concentrate macromolecules at the plasma membrane and, in the case shown here, fuse with lysosomes, where their contents are ***acid-ified, degraded, and trafficked to the *trans* Golgi; bidirectional traffic via recycling endosomes takes place for both endosomes and lysosomes. COPI vesicles mediate trafficking of cargo from the Golgi back to the ERGIC and ER, primarily for retrieval of escaped ER resident proteins. The intestinal pathogens Norwalk virus (NV), poliovirus (PV), *E. coli*, and possibly adenovirus (AdV) -40/41 encode proteins to antagonize various portions of this pathway as indicated. Rotavirus NSP4 also antagonizes vesicle trafficking (not shown).

Table 1

Proteins encoded by gastrointestinal pathogens that antagonize the cellular secretory pathway

Pathogen	Protein	Target	Mechanism	References
<i>E. coli</i>	NleA	COPII vesicle transport	COPII vesicle mislocalization	[13,14•]
	Stx-1	-Unknown-	-Unknown-	[15•]
Norovirus	p22	COPII vesicle transport	COPII vesicle mislocalization via an ER export signal mimic	(Sharp <i>et al.</i> , in preparation)
	NTP	Secretory vesicles?	-Unknown-	[16,17]
Rotavirus	NSP4	Vesicle transport	Inhibition of microtubule-dependent vesicle trafficking	[18]
Enterovirus	3A	COPI vesicle biogenesis	Inhibits GBF1, a co-factor necessary for COPI vesicle biogenesis	[19,20]
	2B	Ion regulation?	Forms pores in cellular membranes to inhibit ion homeostasis	[21–24]
Adenovirus-40/41?	E4orf4	Lysosomes/endosomes	Targets recycling endosomes to the <i>trans</i> Golgi	[25,26]