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Innate host barriers to viral trafficking and population diversity: Lessons learned from poliovirus

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Abstract

Poliovirus is an error-prone enteric virus spread by the fecal-oral route, and rarely invades the central nervous system (CNS). However, in the rare instances when poliovirus invades the CNS, the resulting damage to motor neurons is striking and often permanent. In the pre-vaccine era, it is likely that most individuals within an epidemic community were infected; however, only 0.5% of infected individuals developed paralytic poliomyelitis. Paralytic poliomyelitis terrified the public and initiated a huge research effort, which was rewarded with two outstanding vaccines. During research to develop the vaccines, many questions were asked: Why did certain people develop paralysis? How does the virus move from the gut to the CNS? What limits viral trafficking to the CNS in the vast majority of infected individuals? Despite over 100 years of poliovirus research, many of these questions remain unanswered. The goal of this chapter is to review our knowledge of how poliovirus moves within and between hosts, how host barriers limit viral movement, how viral population dynamics impact viral fitness and virulence, and to offer hypotheses to explain the rare incidence of paralytic poliovirus disease.

I. Introduction

Poliovirus is a non-enveloped enteric RNA virus in the *Picornaviridae* family that the ability to invade the central nervous system (CNS), despite the fact that entering the CNS has no apparent benefit for viral transmission. In the periphery, poliovirus can replicate in many cell types. However, in the CNS, poliovirus replication and subsequent damage is limited to motor neurons. Paralysis from motor neuron damage is often permanent. For this reason, poliovirus has an infamous reputation, and shaped the public view of infectious diseases in the 20th century. In reality, the chances of a person developing serious complications from poliovirus infection are exceedingly small—much smaller than the chances of being seriously injured in an accident. In the pre-vaccine era, it is likely that most individuals within an epidemic community were infected; however, only 4–8% of those infected exhibited any symptoms of disease, with most of these developing a abortive mild febrile illness and nothing more (Pallansch and Roos, 2001). A small subset of those with symptoms developed aseptic meningitis, which was generally self-limiting. Only 0.5% of infected individuals developed paralytic poliomyelitis. Nonetheless, poliovirus terrified the public and launched a massive research effort that rivals that of current HIV research. These efforts were rewarded with two outstanding vaccines-- the Salk inactivated vaccine, and the Sabin live-attenuated vaccine. Due to the overwhelming success of the Salk and Sabin vaccines, poliovirus is no longer a public health threat in developed countries. In 1988, the World Health Organization began a campaign to eradicate poliovirus from the planet by the year 2000, using the live-attenuated trivalent Sabin poliovirus vaccine. Although the eradication attempt has been largely successful, wild- and vaccine-derived poliovirus cases are still being reported in developing countries (Arita, Nakane, and Fenner, 2006; Roberts, 2006a; Roberts, 2006b). There are several reasons for this potential failure, including the high proportion of asymptomatic carriers, lack of complete vaccine coverage in politically

unstable regions, low immunity among some populations despite multiple vaccinations, and reversion of the attenuated vaccine strains. Additional research and resources will be required to eradicate poliovirus.

The benefit of poliovirus research in an era of eradication

Some might question the utility of poliovirus research in an era where the virus has been eradicated from developed countries and global eradication efforts are ongoing. Why study poliovirus in 2010 and beyond?

1. Aid the eradication campaign—Wild poliovirus and poliomyelitis remains endemic to several countries and is spreading. Until recently, wild poliovirus was circulating in just four nations, Afghanistan, Nigeria, Pakistan, India; however, new cases have emerged in several other countries. Additionally, the viruses within the Sabin live-attenuated vaccine frequently revert attenuating mutations, occasionally resulting in cases of vaccine-associated paralytic poliomyelitis (Kew et al., 1981; Nkowane et al., 1987). These revertant viruses can circulate and cause additional disease. Understanding poliovirus evolution and limiting reversion of attenuating mutations will aid eradication efforts.

2. Serve as a model for vaccine development—A long-standing question is why multiple successful vaccines were readily developed for poliovirus when other viruses resist vaccine development. Perhaps by understanding how and why the poliovirus vaccines work, other vaccine endeavors will be more successful.

3. Serve as a model for other enteric viruses—Many viruses, including several medically important enteric viruses, are difficult to study because of limited replication in cell culture and animal models. The robust growth of poliovirus, paired with tools developed over a century of research, make poliovirus an attractive model system for understanding the basic biology of other viruses.

4. Prepare for viruses that will occupy the niche vacated by poliovirus in the post-eradication era—Humans have coexisted with poliovirus for millennia, in a largely stable, minimally pathogenic relationship. It is possible that poliovirus eradication will open a niche to be occupied by another pathogen. Likely candidates include other enteroviruses in the *Picornaviridae* family. Knowledge gained from poliovirus research will inform efforts to guard against future epidemics.

5. Understand other enteroviruses—Poliovirus has lifestyle similar to other occasionally pathogenic enteroviruses. Enteroviruses include poliovirus, echovirus, coxsackievirus, and human enterovirus, and cause 1 billion infections per year worldwide (Oberste et al., 2000). Apparent disease is relatively rare, but enteroviruses account for 5–10 million symptomatic infections per year in the United States (Strikas, Anderson, and Parker, 1986), and are the leading cause of the most common CNS infection, aseptic meningitis. Of particular concern are enterovirus 71 (EV71) and coxsackievirus B3 (CVB3). EV71 is the most significant neurotropic enterovirus in some areas of the world and is likely to be the most important neurotropic/paralytic enterovirus upon eradication of poliovirus (McMinn, 2002) (Chumakov et al., 1979) (Pallansch and Roos, 2001; Shindarov et al., 1979). CVB3 can cause paralysis, myocarditis, and may lead to the development of type I diabetes (Grist, Bell, and Assaad, 1978; Pallansch and Roos, 2001). Over 3% of CVB3 infections result in cardiac signs or symptoms (Grist, Bell, and Assaad, 1978). However, most enterovirus infections are asymptomatic, therefore, understanding how poliovirus moves within a host to initiate disease will shed light on the trafficking and disease course of other enteroviruses.

II. Poliovirus route and barriers

A. Phase I: Gastrointestinal tract

1. Poliovirus in the gastrointestinal tract—In order to propagate itself, poliovirus must be shed from a primary host, survive in the environment, and initiate a productive infection in the gastrointestinal tract of a secondary host. Most infected individuals shed poliovirus in their feces for 2–8 weeks after infection (Nathanson, 2008). Intra-family transmission is rapid and relatively complete (Pallansch and Roos, 2001). Many poliovirus infections are transmitted by direct person-to-person contact, without the need for long-term viral survival in the environment. However, poliovirus infections are also initiated by virus in the environment, via fomites, etc. Although the precise amount of virus required for natural infection is not known, experiments with the Sabin vaccine viruses suggested that 100 viable virions (tissue culture infectious dose units) can productively infect infants (Minor et al., 1981).

After ingestion, poliovirus must bind to and enter cells via its cellular receptor, CD155 (also called PVR, for poliovirus receptor), which is a major susceptibility factor (Mendelsohn, Wimmer, and Racaniello, 1989). While the normal function of CD155 is not completely understood, it is ubiquitously expressed in humans and is involved in the formation of adherens junctions and NK cell recognition (Racaniello, 2006). Importantly, not all tissues that express CD155 are targets for poliovirus replication, and some tissues with low CD155 expression, such as the intestine, are known sites of poliovirus replication (Bernhardt et al., 1994) (Solecki et al., 1997) (Ren and Racaniello, 1992a; Ren et al., 1990). The importance of human CD155 as a susceptibility factor is highlighted by the finding that mice are not susceptible to poliovirus infection unless they express a human CD155 transgene (Koike et al., 1991; Ren et al., 1990). CD155-transgenic mice are susceptible to poliovirus infection and paralytic disease from injected virus; however, they are not susceptible by the natural oral route of infection. The initial explanation for the oral insusceptibility of CD155-transgenic mice was that CD155 expression was not detected in the gastrointestinal tract (Koike et al., 1991; Ren et al., 1990). Several efforts to create orally susceptible mice by using alternate promoters to drive CD155 expression were unsuccessful (Ida-Hosonuma et al., 2002; Zhang and Racaniello, 1997) (Crotty et al., 2002; Yanagiya et al., 2003), despite detectable expression of CD155 in intestinal tissue (Crotty et al., 2002; Yanagiya et al., 2003), and binding of virus to intestine fragments *ex vivo* (Zhang and Racaniello, 1997). These results indicate that CD155 expression is one factor of many required for murine infection by the oral route.

After poliovirus enters the mouth, it may replicate in the oropharynx before transit to the lower gastrointestinal tract (Bodian and Horstmann, 1965; Melnick, 1996; Sabin, 1956). The cell types infected in the oropharynx are not known, but may include lymphatic tissues. While poliovirus undergoes robust replication in the oropharynx of chimpanzees (Sabin, 1956), replication in the human throat appears to be limited, and virus is rapidly cleared, despite long-term replication in the intestine (Bodian, 1952; Sabin and Ward, 1941b). These data imply that viral replication in the oropharynx may be limited and transient in humans.

Productive infection requires viral survival in the stomach and access to and replication within susceptible cells in the intestine; however, the identity of the infected cell type remains unclear. The intestinal mucosa is composed of several different cell types. Epithelial cells, including enterocytes, Paneth cells, and goblet cells, create a barrier that limits microbial translocation while allowing absorption of nutrients from the lumen. Subepithelial immune cells such as intraepithelial lymphocytes and lamina propria lymphocytes, including IgA-producing B cells, play important roles in controlling commensal and pathogenic microorganisms. The intestinal mucosa contains regions of specialized lymphatic tissues

called Peyer's patches. Within Peyer's patches, specialized M cells within the epithelium sample gut antigens and present them to underlying immune cells, which can circulate via the lymphatic system. Virus may initially enter and replicate in intestinal lymphatic tissues or adsorptive epithelial cells (enterocytes)(Figure 1). While there is evidence for viral infection of each cell type, more evidence appears to be in favor of initial infection via cells of the lymphatic system. First, poliovirus was visualized and isolated from human and chimpanzee lymphatic tissue (Bodian, 1955a; Bodian and Horstmann, 1965; Sabin and Ward, 1941a; Sabin and Ward, 1941b) as well as mouse lymphatic tissue following intraperitoneal injection (Buisman et al., 2003). Second, poliovirus injected directly into the ileum of rhesus macaques was visualized in M-like cells, the lamina propria, and within lamina propria macrophages (Takahashi et al., 2008). Third, poliovirus was visualized in M cells after incubation of virus with human tissue containing Peyer's patches (Sicinski et al., 1990). Fourth, poliovirus was exocytosed through M-like cells in culture systems containing monolayer cultures with M cells from human Peyer's patches (Ouzilou et al., 2002). Fifth, poliovirus can replicate in isolated lymphoid cells such as dendritic cells and macrophages, particularly after cellular activation (Eberle, Nguyen, and Freistadt, 1995; Freistadt and Eberle, 1996; Freistadt, Fleit, and Wimmer, 1993) (Wahid, Cannon, and Chow, 2005; Willems, Melnick, and Rawls, 1969). Despite this evidence, it is unknown whether poliovirus actively replicates in lymphoid tissues *in vivo*, or whether it simply drains there. Evidence for viral replication in the superficial epithelium includes a poliovirus strain (Leon) that did not appear to replicate in lymphoid tissues but nonetheless replicated in the gut (Sabin, 1955; Sabin, 1956) and visualization of poliovirus in microvilli epithelial cells, but not M cells, within the intestine of CD155-transgenic mice (Ohka et al., 2007).

The cellular origin for viral shedding and transmission is also unclear. While virus may initially enter and replicate in intestinal lymphatic tissues, gut epithelial cells are candidates for viral shedding into the lumen (Nathanson, 2008). CD155 is localized primarily on the basolateral surface of polarized human CaCo-2 cells, therefore, poliovirus may access epithelial cells from the back, replicate, and be shed apically into the lumen (Boot et al., 2003; Ohka and Nomoto, 2001). This model is supported by data from the Cutter incident, where children were administered incompletely inactivated poliovirus by intramuscular injection. Perhaps predictably, several of these children developed paralytic poliomyelitis; however, contacts of these children were also infected with poliovirus, with 113 developing paralytic disease (Nathanson and Langmuir, 1963a; Nathanson and Langmuir, 1963b; Offit, 2005). Since most of these secondary infections were through the fecal-oral route, virus was able to travel from the injection site to the intestinal tract of the vaccine recipient, with productive viral replication and shedding. Therefore, it is likely that virus accessed the gut lumen by entering the basolateral surface of the epithelium in the vaccine recipients. Experiments with human volunteers suggest that viral replication in the lower gut is required for productive shedding in feces (Sabin, 1956). Overall, it is possible that poliovirus entry and replication in the gut is similar to that of reovirus. Reovirus enters M cells overlying Peyer's patches, enters intestinal epithelial cells from the basolateral surface, replicates, and is shed into the lumen (Bass et al., 1988; Rubin, Kornstein, and Anderson, 1985) (Wolf et al., 1983) (Wolf et al., 1981) (Excoffon et al., 2008). More work is required to identify the specific cell types infected by poliovirus in the gut.

2. Host barriers in the gastrointestinal tract—Poliovirus must navigate several hurdles to initiate productive infection in the gut. First, virus must survive the inter-host period and resist inactivation from desiccation, alternate temperatures and pH, etc. It is thought that poliovirus cannot survive in the environment for more than a couple of weeks, but under optimal conditions of neutral pH, sufficient moisture, and low temperature, the virus may survive for months (Pallansch and Roos, 2001; Tyler and Nathanson, 2001). Many instances of poliovirus transmission are through direct person-to-person contact rather

than exposure through environmental sources such as sewage. These person-to-person transfers are likely to be much more efficient than infection by exposure to environmental sources of virus.

Once poliovirus enters the body through the mouth, several non-specific host barriers are encountered. Saliva, mucus, and cilia may aid viral clearance prior to replication. Passage through the stomach may also pose a challenge to the virus (Salo and Cliver, 1976). The stomach pH is 2.0 or lower, and virus must survive stomach acid prior to reaching the small intestine, where pH ranges from 6.5–7.5 (Ewe et al., 1999). A recent study examined the sensitivity of poliovirus to stomach acid in a murine model and *in vitro*. The results suggest that poliovirus is inactivated by stomach acid after prolonged incubation. When virus was delivered to fasted mice by a gastric tube, little of the inoculum was found in small intestine, although recovery was aided if a pH-neutralizing agent was co-administered with the virus (Ohka et al., 2007). In a different study, CD155-transgenic mice were orally inoculated with a pool of ten marked viruses to identify host barriers and viral bottleneck effects (Figure 2). While all ten viruses were often found in feces, only one or two marked viruses were found in the CNS (Kuss, Etheredge, and Pfeiffer, 2008). These results suggest that the barrier encountered during transit to the CNS was much more severe than the barrier encountered during passage through the gastrointestinal tract. Although the barrier imposed by the gastrointestinal tract may be less severe than the barrier encountered during trafficking to the CNS, recent experiments from our laboratory suggest that only ~1% of the total inoculum is shed in feces of orally inoculated mice (unpublished data and (Kuss, Etheredge, and Pfeiffer, 2008)).

Stomach acid is not the only barrier encountered in the upper GI tract; the virus must resist inactivation by bile salts and digestive enzymes. In general, enveloped viruses are susceptible to inactivation by bile salts, but non-enveloped viruses such as poliovirus are quite resistant. Proteases are secreted by gastric and pancreatic cells, and enteric viruses are either resistant to cleavage, or use proteases to their advantage. For poliovirus, *ex vivo* exposure to proteases present in gastric contents did not reduce viral titer (Ohka et al., 2007). Viruses in the *Reoviridae* family are cleaved by intestinal proteases, which increase viral infectivity *in vitro* and *in vivo* (Clark et al., 1981; Estes, Graham, and Mason, 1981) (Amerongen et al., 1994) (Bass et al., 1990), suggesting that proteases may actually aid some enteric viruses.

Throughout the gastrointestinal tract, a mucus layer lines the epithelium, and viruses must penetrate this layer to gain access to susceptible cell types below. Mucus overlying gut epithelia exists in two layers. The outer, loosely attached layer is 100 μm thick and the inner, firmly attached layer is 50 μm thick (Atuma et al., 2001). The inner layer is largely devoid of bacteria (Johansson et al., 2008). Together, these layers form a 150 μm viscous barrier, which is 5,000 times the 30 nm diameter of a poliovirus particle. By analogy, this layer would amount to a human swimming through 150 gel-filled American football fields. Several mucin genes have been identified, and mucin proteins are heavily glycosylated, forming a net-like structure (Dekker et al., 2002) (Johansson et al., 2008). Mucins can inhibit enteric viruses such as rotavirus (Chen, Baylor, and Bass, 1993) and protect the host from bacterial pathogens such as *Campylobacter* (McAuley et al., 2007) and *Citrobacter* species (Bergstrom et al.). The precise effect of the mucus layer on viral infection is unknown.

In addition to the barrier function created by mucus, the mucus layer contains secretory IgA, and other secreted factors that may have antiviral activity. Over 75% of immunoglobulin in the body is IgA, and most is secreted across mucus membranes. IgA is important for homeostasis of the gut environment and is impacted by the gut microflora (Macpherson and

Slack, 2007) (Macpherson et al., 2008; Tomasi et al., 1965). For poliovirus, the appearance of poliovirus-specific IgA in the intestine coincides with decline in poliovirus shedding and eventual clearance (Valtanen et al., 2000). Therefore, IgA plays a role in control of poliovirus infections in the gut. As a member of the immunoglobulin superfamily, the poliovirus receptor CD155 has the potential to limit viral infection through a secreted form of the receptor. There are four isoforms of human CD155; two isoforms are membrane bound, and two isoforms are secreted (Baury et al., 2003). The secreted forms of CD155 are detectable in cultured cell medium, human serum, and human cerebrospinal fluid, and have the capacity to limit viral entry by blocking binding to membrane-bound CD155 (Baury et al., 2003). In fact, many viral receptors are members of the immunoglobulin superfamily, and since most viruses bind these proteins at the most membrane-distal site, it has been suggested that soluble forms of viral receptors may be precursors to modern antibodies, and that soluble receptors neutralize viruses by competing with receptors on the cell surface (Dermody et al., 2009). While this idea of soluble receptor-mediated poliovirus neutralization has not been examined *in vivo* and may be operative during human infections, current transgenic mouse models express only the membrane-bound form of CD155, therefore, soluble CD155 is unlikely to contribute to the major host barriers observed in this mouse model (see below)(Koike et al., 1990; Koike et al., 1991; Kuss, Etheredge, and Pfeiffer, 2008; Lancaster and Pfeiffer, 2010; Ren et al., 1990).

A variety of relatively non-specific host defense mechanisms may contribute to enteric virus barriers in the gut lumen. Defensins are host-derived antimicrobial proteins produced by specialized gut epithelial cells (Ayabe et al., 2000) (Salzman, Underwood, and Bevins, 2007). While defensins are generally thought of as antibacterial proteins due to their membrane disruption potential, there is evidence that defensins may target both enveloped and non-enveloped viruses (Gropp et al., 1999) (Buck et al., 2006) (Daher, Selsted, and Lehrer, 1986; Hazrati et al., 2006; Wang et al., 2004b). For non-enveloped viruses such as adenovirus, the antiviral activity of defensins is independent of membrane disruption, and may involve inhibition during intracellular steps in the viral replication cycle (Smith and Nemerow, 2008). Lactoferrin is another relatively non-specific host protein with antibacterial and antiviral properties. Lactoferrin is present in milk, mucosal secretions, and secretory granules of neutrophils, and inhibits bacteria through sequestration of iron. Lactoferrin has antiviral activity for several viruses including rotavirus, enteroviruses, and adenovirus, and is thought to act by inhibiting viral internalization (Seganti et al., 2004) (Weng et al., 2005).

Once a virus reaches the cell surface, it must enter and initiate steps to ensure propagation. It is uncertain which cell types are infected by poliovirus in the gut. If the initial entry event occurs in epithelial cells, it is likely to require expression of the poliovirus receptor, CD155. Viral receptor expression in the intestinal epithelium, M cells, and germinal centers correlates with oral susceptibility (Iwasaki et al., 2002). For poliovirus, humans are the most orally susceptible, followed by chimpanzees, Old World monkeys, New World monkeys, and CD155-transgenic mice (Sabin, 1956) (Iwasaki et al., 2002). While CD155 expression is barely detectable or undetectable in several strains of non-orally susceptible CD155-transgenic mice (Koike et al., 1991; Ren et al., 1990), viral replication can still occur in the gut and replicated virus is shed in feces (our unpublished data) (Boot et al., 2003; Kuss, Etheredge, and Pfeiffer, 2008), suggesting that other host factors contribute to the lack of disease in these mice following oral inoculation (see discussion below on the role of type I interferon, etc.). If the initial entry event occurs via transcytosis through M cells, the process may be CD155-dependent or CD155-independent. M cells sample gut antigens non-specifically, so it is possible that viral entry and transcytosis through M cells may not require the viral receptor. However, poliovirus replication requires CD155, since viral uncoating is triggered by receptor engagement. Studies suggest that binding and entry in M cells may be

very inefficient, since less than 1% of the input virus was transcytosed through M cells in a cell culture model (Ouzilou et al., 2002), and the proportion of poliovirus bound to M cells in *ex vivo* human Peyer's patch tissue was minimal, even compared with reovirus in a similar model (Sicinski et al., 1990; Wolf et al., 1981). Overall, the process of viral entry into gut tissues is likely to be inefficient, constituting a major barrier for viral replication.

The host's innate immune response through induction of interferons and interferon-induced genes is a major impediment to viruses. Viral replication generates a variety of pathogen-associated molecular pattern molecules, such as double-stranded RNA, that are detected by the host and lead to induction of a variety of antiviral effectors. Key components of this pathway are type I interferons, interferon α and β , which are produced by many cell types in response to viral infection. IFN α/β are released from infected cells and bind the interferon α/β (IFNAR) receptor on neighboring cells, which are protected by an induced antiviral state. The importance of IFNAR in limiting many viruses has been demonstrated in experiments with mice lacking the IFNAR gene (Fiette et al., 1995; Garcia-Sastre et al., 1998; Mrkic et al., 1998; Muller et al., 1994; Ryman et al., 2000; Samuel and Diamond, 2005; Shresta et al., 2004; Wessely et al., 2001). IFNAR knockout mice demonstrate increased susceptibility to a wide variety of viruses, including poliovirus. In fact, CD155-transgenic mice are not orally susceptible unless they lack IFNAR expression, indicating the importance of the type I interferon pathway in protecting the host (Ida-Hosonuma et al., 2005; Ohka et al., 2007). Interestingly, poliovirus can replicate in the gut of CD155 mice that have functional IFNAR, suggesting that the inhibitory effect of interferon may act at a post-gut step (see below) (Boot et al., 2003) (our unpublished data). The role of IFN α/β in limiting viral replication in the periphery will be discussed in more detail in the next section. Recently, type III interferon (a.k.a. IFN λ or IL-28/29) has emerged as an important contributor to host innate immune responses, particularly at mucosal surfaces (Kotenko et al., 2003) (Sheppard et al., 2003). The response of IFN λ to infection with several viruses, including Theiler's murine encephalitis virus and mouse hepatitis virus, is particularly high in stomach, intestine, and lung, and appears to protect epithelial surfaces (Sommerey et al., 2008) (Mordstein et al., 2010).

The gut architecture and natural homeostasis may be another obstacle to enteric viruses. Epithelial cells are constantly sloughing and being shed from villi. Therefore, enteric viruses using epithelial cells for replication and shedding must constantly infect new cells to maintain prolonged shedding (Boshuizen et al., 2003). Cells of the gastrointestinal tract are joined by tight junctions that prevent passage of pathogens and commensals. Viruses requiring access to lymphatics or blood must penetrate this impressive barrier. Using a pool of ten marked polioviruses, it was observed that most viruses in the gut lumen are unable to access gut tissues or pass the gut barrier and enter the bloodstream and/or CNS (Kuss, Etheredge, and Pfeiffer, 2008). However, if the gut epithelium was disrupted with dextran sulfate sodium treatment prior to oral poliovirus infection, more of the marked viruses were observed in gut tissues and blood. Interestingly, gut damage was unable to allow more viruses in the population to reach the CNS, suggesting that the gut epithelium is one barrier of several which must be overcome for poliovirus transit to the CNS (Kuss, Etheredge, and Pfeiffer, 2008).

B. Phase II: Blood and peripheral tissues

1. Poliovirus in blood and peripheral tissues—Following primary viral replication in the mucosa, it is thought that poliovirus drains into cervical and mesenteric lymph nodes, and then into blood. Virus is only detectable in the blood if the rate of viral input due to replication exceeds the rate of clearance by the reticuloendothelial system and other mechanisms. Transient viremia is common during poliovirus infections, and this primary

viremia may occur in most people and orally infected chimpanzees (Horstmann, Mc, and Mascola, 1954; Horstmann et al., 1954) (Melnick et al., 1961) (Bodian, 1952). A subset of infected individuals, roughly 4–8%, develop a secondary, major viremia due to continued viral replication in extraintestinal sites (Bodian and Paffenbarger, 1953; Horstmann, Mc, and Mascola, 1954; Horstmann et al., 1954) (Bodian and Horstmann, 1965; Melnick, 1996) (Sabin, 1956). The secondary, major viremia is often accompanied by symptoms of minor illness, and is a prerequisite for the development of CNS disease. At this stage, virus is thought to replicate in non-neural sites including brown fat, reticuloendothelial tissue, and muscle (Bodian, 1955a) (Wenner and Kamitsuka, 1957) (Ren and Racaniello, 1992a; Sabin, 1956).

2. Host barriers in blood and peripheral tissues—The three major barriers limiting poliovirus during the blood and peripheral tissue stage are clearance by the reticuloendothelial system, innate immune responses, and adaptive immune responses. Even prior to extraintestinal replication or development of an adaptive immune response, virus is cleared by phagocytic cells of the reticuloendothelial system. For other viruses such as yellow fever virus and herpes simplex virus, inhibition of macrophage phagocytosis increases viremia (Irie et al., 1998) (Tyler and Nathanson, 2001). Phagocytic clearance is impacted by the size of the particle; therefore, opsonization of viruses with antibody or complement increases efficiency.

Innate immune responses are critical components of the host's defense against viral infections, and for poliovirus, the innate immune response primarily limits viral replication during the blood-extraintestinal tissue phase. Poliovirus and other enteric picornaviruses are sensitive to type I interferon *in vivo* (Fiette et al., 1995) (Wessely et al., 2001) (Ida-Hosonuma et al., 2005; Yoshikawa et al., 2006). As described above, CD155 transgenic mice are not orally susceptible to poliovirus unless they have limited IFN α/β responses due to IFNAR gene deletion (Ohka et al., 2007). Additionally, poliovirus replication is enhanced in CD155-expressing-IFNAR knockout mice, with viral replication and high viral titers in tissues, such as liver, pancreas, and kidney, which are not normally sites of replication in immune-competent mice (Ida-Hosonuma et al., 2005; Yoshikawa et al., 2006). Poliovirus susceptibility in various tissues correlates with amount of interferon induction and levels of interferon-stimulated genes prior to infection (Ida-Hosonuma et al., 2005; Yoshikawa et al., 2006) (Ohka et al., 2007). These results prompted Ida-Hosonuma et al. to propose that visceral organs of immune competent mice, which are more likely to encounter microbes leaking from the intestinal tract, are primed for a fast response to infection, while CNS tissues are not (Ida-Hosonuma et al., 2005). While a pool of ten marked polioviruses was severely restricted during travel to the CNS of immune competent CD155-transgenic mice after intraperitoneal or intramuscular injection, this restriction was dramatically diminished in CD155-IFNAR knockout mice (Kuss, Etheredge, and Pfeiffer, 2008; Lancaster and Pfeiffer, 2010). Taken together, these studies highlight the importance of innate immune responses, particularly type I interferon, in restricting poliovirus spread in the periphery.

The best-known barrier limiting poliovirus viremia and the development of disease is neutralizing antibody. Decades of research have shown that neutralizing antibody protects humans and primates from paralytic disease by preventing viremia (Nathanson, 2008). Passive antibody delivered prior to infection is protective, and there is a strong correlation between poliovirus antibody titer and protection from paralysis (Bodian, 1953; Bodian, 1955a; Sabin, 1956). However, serum antibodies do not prevent viral replication in the gut, since humans vaccinated with inactivated virus via injection still allow replication of the live attenuated vaccine after oral delivery (Horstmann, 1955; Sabin, 1956). Children vaccinated with the live attenuated vaccine become infected after challenge with a second dose, but they shed less virus over a shorter time course, suggesting that poliovirus-specific IgA may

limit viral replication in the gut (Horstmann, 1955; Sabin, 1956). During primary infections, clearance of viremia is immediately followed by detectable serum antibody specific for poliovirus (Nathanson, 2008). The efficacy of the inactivated/injected poliovirus vaccine suggests that serum IgG is sufficient to prevent paralysis; however, the additional mucosal IgA induced by the live attenuated/oral vaccine may at least partially limit viral replication in the gut and subsequent shedding. The dramatic success of the Salk and Sabin poliovirus vaccines is proof that neutralizing antibodies constitute a major host barrier to viral replication, spread, and CNS invasion.

C. Phase III: CNS

1. Poliovirus access to the CNS—Poliovirus rarely invades the CNS, either through the blood-brain barrier or trafficking in neurons. CNS infection and subsequent damage is an accidental diversion from the gastrointestinal tract, with apparently little benefit to the virus. While poliovirus can undergo robust replication in the CNS and generate high viral titers, CNS virus is not likely to be transmitted to a new host by the normal fecal-oral route. The route of viral CNS entry has been debated for decades, with evidence for both blood and neural transit routes. Regardless of the CNS entry mechanism, it has been established that viremia must precede viral CNS invasion.

a. Poliovirus access to the CNS via the blood-brain barrier: The blood-brain barrier is composed of microvasculature containing tight junctions with dense basement membranes, such that an active transport or carrier system is required for passage through these specialized capillaries. Some viruses, including West Nile virus and adenovirus, breach the blood-brain barrier to access the CNS (Dai et al., 2008; Gralinski et al., 2009; Wang et al., 2008; Wang et al., 2004a). This blood-brain barrier disruption can be observed by influx of dyes normally sequestered from the CNS. Poliovirus infection does not appear to induce blood-brain barrier disruption (Yang et al., 1997), and Evan's blue dye does not enter the brain in murine infection models (our unpublished data). Yang et al. examined the ability of poliovirus to enter the CNS via the blood route using ³⁵S-labeled virions in intravenously-injected mice (Yang et al., 1997). Poliovirus was observed in many tissues, and although the overall amount of virus in the cerebrum and cerebellum was the lowest, it was much greater than the theoretical amount predicted based on the vascular volume of the brain. Importantly, the amount of labeled virus in the brain at 5 hours post-infection was similar in CD155-transgenic and non-transgenic mice, suggesting that brain access was viral receptor-independent (Yang et al., 1997). However, in this study, the brains were not perfused prior to radioactive counting, and due to the high particle:plaque-forming unit ratio of poliovirus, it is uncertain whether viruses found in the brain are functional. Nonetheless, this study provided precedent that poliovirus entry into the CNS via the blood route can be modeled in mice.

Poliovirus may use a variety of mechanisms to access the CNS through the blood-brain barrier. First, the CNS contains regions with reduced blood-brain barrier strength. In these sites, such as the area postrema in medulla oblongata, the capillary endothelium lacks tight junctions, which may allow more efficient transport of viruses (Bodian, 1952; Tyler and Nathanson, 2001). Second, it has been proposed that picornaviruses can directly infect endothelial cells (Blinzinger et al., 1969; Couderc et al., 1990) (Zurbriggen and Fujinami, 1988) (Friedman et al., 1981). Third, virus entry into the CNS may be aided by factors that increase vascular permeability (Sellers, 1969). Fourth, some viruses, such as measles and mumps, use transendothelial transport via diapedesis of immune cells. A "Trojan horse" mechanism for poliovirus transport in macrophages has been proposed, but not yet proven experimentally (Freistadt, Fleit, and Wimmer, 1993).

b. Poliovirus access to the CNS via neurons: Like many other viruses, poliovirus can infect neurons and undergo axonal transport. Infection of neurons and transport mechanisms have been investigated for a variety of viruses including herpesviruses (Smith, Gross, and Enquist, 2001) (Card et al., 1991), rabies (Iwasaki and Clark, 1975), reovirus (Morrison, Sidman, and Fields, 1991), West Nile virus (Samuel et al., 2007), adenovirus (Salinas et al., 2009), and picornaviruses (Martinat et al., 1999). Early evidence for poliovirus infection of neurons was generated in primate studies in the 1930s, 1940s, and 1950s (Bodian and Howe, 1940) (Fairbrother and Hurst, 1930) (Howe and Bodian, 1942) (Faber, Silverberg, and Dong, 1953) (Verlinde, Kret, and Wyler, 1955). Poliovirus was found in nerve fibers after intramuscular, intravenous, or oral infection of monkeys. However, the role for viral transport in neurons was debated since nerve freezing or transection blocked viral transport in some studies, but not others (Howe and Bodian, 1941) (Bodian, 1954) (Nathanson and Bodian, 1961) (Racaniello, 2006). These and other data sparked debate about the mechanism of poliovirus CNS entry, which is still not completely understood today.

CD155-transgenic mice have been a useful tool for examining poliovirus neural transport mechanisms. Unlike in monkeys, in CD155-transgenic mice, there is consensus that poliovirus travels to the CNS via the neural route after intramuscular injection. In this mouse model, the injected limb is always the first limb paralyzed, and sciatic nerve transection protects mice from paralytic disease following intramuscular injection in the leg (Ohka et al., 1998; Ren and Racaniello, 1992b). It is thought that virus enters the sciatic nerve at the neuromuscular junction (Ohka et al., 2004), travels up the sciatic nerve to the spinal cord, and is then transported to the brain. Sciatic nerve transection experiments have revealed that poliovirus is transported by the fast retrograde axonal transport system, at a rate of over 12 cm/day (Ohka et al., 2009; Ohka et al., 1998). Transport in the sciatic does not require replication in muscle (Lancaster and Pfeiffer, 2010; Ohka et al., 1998), and intact 160S viral particles are observed in the sciatic nerve (Arita et al., 1999; Ohka et al., 2009; Ohka et al., 1998). Perhaps not surprisingly, poliovirus does not undergo replication in long axons of the sciatic nerve (Lancaster and Pfeiffer, 2010). The cytoplasmic tail of the viral receptor CD155 interacts with Tctex-1 (Tctel-1), a light chain of cytoplasmic dynein (Ohka et al., 2004) (Mueller et al., 2002). Taken together, these data suggest a model where poliovirus enters neurons at the neuromuscular junction via receptor-mediated endocytosis, the cytoplasmic tail of the receptor binds the dynein motor complex, and the virus-containing endosome is transported on microtubules to the cell body via the fast retrograde axonal transport system (Figure 3). Uncoating and replication occur in the neural cell body, which can be very distant from the viral point of entry at the neuromuscular junction. Poliovirus entry and uncoating in neurons is very different from that in HeLa cells, where viral uncoating takes place in close proximity to the plasma membrane (Brandenburg et al., 2007). Therefore, neurons represent a unique environment, very distinct from cells found in the gut and periphery.

2. Host barriers en route to the CNS—Regardless of whether poliovirus enters the CNS via a blood or neural route, several barriers may limit viral access. Poliovirus variants found in the CNS of human vaccine-associated paralytic poliomyelitis patients corresponded to only a small subset of viruses found in the gut, suggesting a barrier limited CNS access (Georgescu et al., 1997; Georgescu et al., 1994). Similarly, when CD155-transgenic mice were orally inoculated with a pool of ten marked polioviruses, only a small subset were found in the CNS (Kuss, Etheredge, and Pfeiffer, 2008; Lancaster and Pfeiffer, 2010). Therefore, regardless of the mechanism of CNS entry, following oral infection, viruses found in the CNS represent a small subset of the peripheral population, suggesting the existence of one or multiple host barriers limiting CNS access.

Several viruses disrupt the blood-brain barrier, suggesting that it can impede viral transit in the absence of viral intervention (Soilu-Hanninen et al., 1994) (Toborek et al., 2005) (Wang et al., 2004a). In fact, a non-neuroinvasive variant of West Nile virus can enter the brain only if the blood-brain barrier was artificially breached (Kobiler et al., 1989). Despite the fact that poliovirus enters the CNS of CD155-transgenic mice from the blood (Yang et al., 1997), there is not evidence to suggest that poliovirus disrupts the blood-brain barrier. Therefore, the blood-brain barrier may impede poliovirus access to the CNS.

Viral trafficking to the CNS via the neural route is subject to several barriers. First, viral infection can induce innate immune responses in neurons. Several viruses, including West Nile virus, herpesviruses, and rabies virus, induce production of type I interferon and other innate immune effectors in neurons (Prehaud et al., 2005) (Delhay et al., 2006) (Griffin, 2003; Samuel et al., 2006). Second, substrates for viral replication are limited in axons. Poliovirus replication requires ribosomes, membrane vesicles derived from intracellular membrane compartments, and other factors that are unlikely to be plentiful in long axons or even nerve terminals. Therefore, viral replication is likely to be limited to neural cell bodies. Two lines of evidence suggest that poliovirus replication does not occur in long axons using a sciatic nerve model in CD155-transgenic mice: the presence of intact 160S particles (Arita et al., 1999; Ohka et al., 2009; Ohka et al., 1998), and lack of viral replication scored by dye-containing virions (Lancaster and Pfeiffer, 2010). Third, viral trafficking in long axons is inefficient. Using a pool of marked polioviruses in a CD155-transgenic mouse model, only a subset of the input population was observed in the CNS following intramuscular inoculation (Kuss, Etheredge, and Pfeiffer, 2008; Lancaster and Pfeiffer, 2010; Pfeiffer and Kirkegaard, 2006). Sciatic nerve sectioning revealed that, of the input population injected into the leg muscle, ~80% was in the lower section of the sciatic nerve, ~50% was in the middle section of the sciatic nerve, and only ~20% was in the upper section of the sciatic nerve (Lancaster and Pfeiffer, 2010). These results suggest that retrograde axonal transport of poliovirus is inefficient, which may contribute to the low disease incidence from poliovirus infections. Studies with cultured neurons may aid our understanding of poliovirus transport barriers in neurons. However, a recent study demonstrated that cultured neurons do not completely recapitulate all transport mechanisms observed in mouse models (Ohka et al., 2009).

Clues to CNS transport mechanisms and potential barriers have come from the phenomenon of “provocation poliomyelitis”, wherein increased poliovirus CNS disease follows peripheral tissue damage (McCloskey, 1950) (Strebel et al., 1995). This tissue damage can be in the form of physical exertion, trivial muscle injury, or injections near the time of poliovirus infection. For example, trauma increased the incidence of paralysis in the injured limb, in naturally infected humans as well as in primate and mouse models (Hill and Knowelden, 1950) (Bodian, 1954) (Bodian, 1955a) (Gromeier and Wimmer, 1998; Ohka et al., 1998; Ren and Racaniello, 1992b) (Lancaster and Pfeiffer, 2010). Similarly, injections administered within 30 days of the oral attenuated poliovirus vaccine or during poliovirus epidemics increased the incidence of paralytic disease (Strebel et al., 1995) (Anderson and Skaar, 1951) (Guyer et al., 1980) (Hill and Knowelden, 1950) (McCloskey, 1950). The “provoking” effect could be acting through a variety of mechanisms including localized alteration of the blood-brain barrier at corresponding areas of the CNS (Bodian, 1954; Bodian, 1955a; Bodian, 1955b; Sutter et al., 1992), inflammation, up-regulation of the viral receptor (Nieke and Schachner, 1985; Squitti et al., 1999), increased viral replication, or increased efficiency of retrograde axonal transport (Gromeier and Nomoto, 2002). Peripheral injury may increase permeability of the corresponding CNS region (Trueta, 1955), but this mechanism has never been experimentally validated for poliovirus CNS access. There is evidence to suggest that muscle damage increases the efficiency of poliovirus retrograde axonal transport. Needle sticks delivered to intramuscularly injected

CD155-transgenic mice increased the proportion of ten marked viruses able to traffic to the upper sciatic nerve and spinal cord (Lancaster and Pfeiffer, 2010). This muscle damage also increased the retrograde axonal transport efficiency of a non-viral protein, suggesting a general mechanism that may be involved in sensing tissue damage by relaying information to and from the cell body (Lancaster and Pfeiffer, 2010). The fact that tonsillectomy increased the incidence of bulbar/brainstem poliomyelitis supports the idea that damage to human tissue may increase neural transport of poliovirus (Modlin, 1995; Mueller, Wimmer, and Cello, 2005). Overall, provocation poliomyelitis highlights the inefficiency of poliovirus CNS access in the absence of damage. Figure 4 contains a summary of poliovirus trafficking routes and potential host barriers discussed in this chapter.

III. Effects of the host barriers on viral population dynamics

Host barriers limit viral population diversity, and viral population diversity is hypothesized to be important for viral fitness and virulence. Therefore, in addition to their established role in limiting viral movement and replication, host barriers may also limit viral fitness through population bottleneck effects. This section describes the effect of host barriers on viral population dynamics and fitness.

A. RNA virus mutations

The RNA-dependent RNA polymerases (RdRps) of poliovirus and many other RNA viruses are error-prone, giving rise to the highest mutation rates known in nature (Drake, 1993) (Domingo and Holland, 1997). Observed error rates vary for different viruses and assays, but most measurements place the error frequency of RNA viruses at approximately 1 error per 10,000 nucleotides copied (Drake et al., 1998) (Domingo et al., 1996; Domingo and Holland, 1997; Domingo et al., 1978; Mansky and Temin, 1995). This translates into approximately one error per genome per cycle of replication. The errors incorporated by viral RdRps can have several consequences for the virus: the vast majority of mutations are deleterious, some mutations are neutral, and occasionally, under the right selective conditions, a few mutations are beneficial. Under selective pressure, mutations conferring resistance to neutralizing antibodies (van Doorn et al., 1995), cytotoxic T cell epitopes (Timm et al., 2004) (Weiner et al., 1995), and anti-viral drugs (Mansky, 2002) can emerge and take over the viral population, despite often having lower fitness than the parent virus in the absence of the selective pressure. Additionally, mutations that aid trafficking within the host may be required for virulence. RNA viruses are often referred to as “quasispecies”, a theoretical concept developed by Eigen and Schuster to describe complex populations (Eigen, 1996). Under certain conditions, RNA viruses are the best real-world example of the theoretical quasispecies (Domingo and Holland, 1997), and any description of RNA virus pathogenesis must take into account their incredible sequence diversity.

A long-standing hypothesis in population genetics is that RNA viruses have evolved optimal error frequencies, and that too many or too few errors will reduce the fitness of the viral population. Fitness is a measure of the ability to produce infectious progeny in a given environment (Domingo and Holland, 1997). Because of their high error rates, RNA viruses exist near the “error threshold”: the value of fidelity where genetic information is irreversibly lost due to mutation, resulting in reduced fitness (Eigen, 1971). Presumably, RNA viruses risk over-mutagenesis of part of the population to reap potential benefits of enhanced adaptability for another subset of the population (Domingo and Holland, 1997).

B. Fitness loss from excessive mutagenesis

Mutagen passage experiments have provided evidence for fitness loss from over-mutagenesis. Because of their high error rates, RNA viruses are very susceptible to

mutagens, which extinguish the population through “error catastrophe” (Crotty et al., 2000) (Domingo and Holland, 1997). In fact, the entire anti-viral effect of the nucleoside analog drug ribavirin, at least for poliovirus, is due to enhanced mutagenesis of the viral genome (Crotty, Cameron, and Andino, 2001). Other viruses such as vesicular stomatitis virus, human immunodeficiency virus (HIV), foot and mouth disease virus (FMDV), and lymphocytic choriomeningitis virus also undergo lethal mutagenesis upon mutagen treatment (Holland et al., 1990) (Loeb et al., 1999) (Sierra et al., 2000) (Crotty et al., 2000). Mutagen-driven extinction of RNA viruses is a promising anti-viral strategy; however mutagen-resistant isolates can emerge (Pfeiffer and Kirkegaard, 2003) (Arnold et al., 2005; Sierra et al., 2007). Poliovirus passaged in the presence of ribavirin develops resistance to ribavirin and other mutagens via a single amino acid change, G64S, in the polymerase that increases the fidelity of RNA replication (Arnold et al., 2005; Pfeiffer and Kirkegaard, 2003). The complete crystal structure of the poliovirus RdRp revealed mechanistic insights into the enhanced fidelity of this polymerase: the mutation may alter the conformation of the active site, allowing more time for correct NTP discrimination (Arnold et al., 2005; Thompson and Peersen, 2004).

C. Fitness loss from limited viral population diversity

Pathogenesis experiments with high-fidelity, ribavirin-resistant G64S poliovirus have provided evidence for fitness loss due to low diversity (Pfeiffer and Kirkegaard, 2005; Vignuzzi et al., 2006). Although no growth defects were observed for the high-fidelity virus in cell culture, this virus was attenuated in mice (Pfeiffer and Kirkegaard, 2005). The attenuation was at least partly due to the increased fidelity of this virus, since it was less able to revert an additional attenuating mutation to restore virulence under selective pressure during replication in mice (Pfeiffer and Kirkegaard, 2005). Vignuzzi et al. showed the virulence of the high fidelity virus could be restored by pre-passage in mutagen to restore the quasispecies before murine inoculation (Vignuzzi et al., 2006). Viruses with high fidelity RdRps show promise as live attenuated vaccines (Vignuzzi, Wendt, and Andino, 2008). Together, these results suggest that viral population diversity is important for pathogenesis in infected animals, where a variety of selective pressures act on the viral population.

Fitness can also be lost by fixation of deleterious mutations via genetic bottlenecks, and much has been learned about fitness limitations imposed by experimentally applied bottlenecks during viral passage in cell culture. For example, when RNA virus quasispecies diversity is repeatedly limited by *in vitro* serial plaque-to-plaque transfer bottlenecks, the resulting populations are less fit than the parental populations (Chao, 1990) (Clarke et al., 1993) (Duarte et al., 1992) (Escarmis et al., 1996) (Yuste et al., 1999). The amount of fitness loss is dependent on the fitness of the initial population (Duarte et al., 1994) (Domingo et al., 1978). The bottleneck-imposed fitness loss correlates with the loss of consensus, most-fit “wild-type” master sequence. This phenomenon is called “Muller’s ratchet”: when small populations have high mutation rates and most mutations are deleterious, “a kind of irreversible ratchet mechanism” reduces fitness (Muller, 1964). Fitness can sometimes, but not always, be restored by passage at high multiplicities of infection (Duarte et al., 1993; Duarte et al., 1994) (Novella et al., 1995). Additionally, recombination-mediated genome repair can rescue populations from Muller’s ratchet (Felsenstein, 1974) (Nee, 1988). Muller’s ratchet may be important for disease severity when bottlenecks exist during spread to, or spread within, hosts.

Virus populations experience major bottleneck events during intra-host and inter-host spread, and these events can impact the fitness and virulence of the remaining viral population. For example, in serial transmission events in pigs, FMDV experienced intra-host and inter-host bottlenecks and the virus population lost virulence by the 14th passage (Carrillo et al., 1998; Carrillo et al., 2007). GB virus B, a relative of hepatitis C virus

(HCV), experienced bottlenecks within experimentally infected primates, which may have reduced viral fitness (Weatherford et al., 2009). HIV and HCV undergo major bottlenecks during human transmission, with estimates of one or a few variants transmitted to the new host (McNearney et al., 1992) (Haaland et al., 2009) (Laskus et al., 2004). HIV spread within a host may also be restricted, since limited populations were observed in splenic germinal centers (Gratton et al., 2000). Despite these major bottleneck events, HIV and HCV quickly restore population diversity, and remain highly successful pathogens.

Poliovirus populations also experience bottlenecks in mouse models and in humans. Using a pool of ten marked viruses, the poliovirus population was severely restricted in the brain following inoculation by a variety of routes (intramuscular, intravenous, intraperitoneal, and oral) (Kuss, Etheredge, and Pfeiffer, 2008; Lancaster and Pfeiffer, 2010; Pfeiffer and Kirkegaard, 2006). Importantly, virulence, as measured by length of time to disease onset, correlated with viral population diversity in the brain. Mice with a more diverse viral population in the brain developed symptoms of disease significantly faster than mice with a less diverse population in the brain (Kuss, Etheredge, and Pfeiffer, 2008). Overall, these studies suggest that poliovirus populations experience dramatic bottleneck effects due to host barriers. A similar stochastic bottleneck effect is seen in humans with vaccine-derived paralytic poliomyelitis. Analysis of matched samples from stool and cerebrospinal fluid revealed that the CNS virus is just one member of the diverse population found in the gut (Georgescu et al., 1997). Interestingly, the most neurovirulent virus present in the gut was not always the “winner” found in the CNS (Georgescu et al., 1997; Georgescu et al., 1994). When inoculated into mice, the human vaccine-derived strains resulted in stochastic CNS invasion, typical of the bottleneck. Because a diverse viral population is required for full virulence, barriers that restrict viral quasispecies may reduce pathogenesis by limiting viral fitness in addition to limiting CNS entry (Domingo and Holland, 1997; Farci et al., 2002; Kuss, Etheredge, and Pfeiffer, 2008; Pfeiffer and Kirkegaard, 2005; Vignuzzi et al., 2006) (Kimata et al., 1999).

IV. Summary

Despite 100 years of poliovirus research, many questions remain unanswered. The rare incidence of paralytic poliomyelitis may be at least partially explained by barriers that limit viral trafficking within an infected host. Physical, anatomical, physiological, and immunological host barriers limit poliovirus replication, trafficking, and the maintenance of viral population diversity, all of which are required for virulence. Despite these barriers, poliovirus transmission persists, suggesting that the virus has evolved mechanisms to cope with countermeasures of the host. Because host barriers are operative for many viral and bacterial pathogens, understanding host barriers will aid not only the poliovirus eradication initiative, but also efforts targeting other pathogens.

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Figure 1.

Architecture of the gastrointestinal epithelium. Virus may access the epithelium through M cells and the lymphatic system, or epithelial cells. IEL, intraepithelial lymphocyte; LPL, lamina propria lymphocyte; DC, dendritic cell; PP, Peyer's patch.

Figure 2.

Identification of poliovirus barriers using a viral population diversity assay. A. Silent mutations in the capsid-coding region (indicated in bold underline) were used to genetically mark ten polioviruses such that host barriers and viral population bottlenecks could be identified in an orally susceptible poliovirus mouse model (CD155^{+/+}IFNAR^{-/-}). B. Following infection and isolation of total RNA, the tag region was amplified by RT-PCR, blotted on a membrane, and probed with ³²P-labeled oligonucleotides matching each tag sequence. C. Each probe hybridized specifically with its cognate virus. D. Samples were harvested from mice orally inoculated with the ten-virus pool upon disease onset, and data from the hybridization assay revealed host barrier-mediated viral population restriction during trafficking within the host. Data from two representative mice are shown. AA, amino acid; WT PV, wild-type poliovirus sequence; Sm. Int., small intestine. Data are from (Kuss, Ethredtge, and Pfeiffer, 2008).

Figure 3.

Model for poliovirus trafficking in neurons. Poliovirus engages CD155 at the nerve terminal and is endocytosed. The cytoplasmic tail of CD155 interacts with a component of the dynein complex, and the virus-containing endosome moves through retrograde axonal transport to the cell body. Once in the cell body, viral uncoating and replication occur.

Figure 4.

Summary of poliovirus trafficking routes and potential host barriers. In most infections, virus follows the course indicated by the bold arrows, entering the mouth, replicating in the intestinal mucosa, with shedding in feces. Virus can also enter the blood. Rarely, virus accesses the CNS through blood or neural routes. Potential host barriers limiting viral movement at each stage are indicated by grey boxes. BBB, blood-brain barrier; IFN, interferon; Ig, immunoglobulin; CNS, central nervous system; ENS, enteric nervous system (i.e., vagus nerve).