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One hundred years of poliovirus pathogenesis

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Abstract

Poliovirus was first isolated nearly 100 years ago in a landmark experiment that established the viral etiology of poliomyelitis. This discovery stimulated investigation of the pathogenesis of poliomyelitis in many laboratories. Nearly 50 years later, when two effective poliovirus vaccines were developed, the impetus to study poliovirus pathogenesis waned. The identification of the cell receptor for poliovirus, CD155, and its use in the development of transgenic mice susceptible to poliovirus revived interest in understanding how the virus causes disease. Experiments in CD155 transgenic mice have provided new information on the initial sites of virus replication in the host, how the virus spreads to the central nervous system through the blood and by axonal transport, the determinants of viral tropism, and the basis for the attenuation phenotype of the Sabin vaccine strains. Despite these advances, our understanding of poliovirus pathogenesis is still incomplete. The dilemma is not how to answer the remaining questions, but whether there will be sufficient time to do so before global eradication of poliomyelitis leads to cessation of research on the disease.

Contents

Introduction

Near the beginning of the 20th century epidemics of the acute central nervous system disease known as poliomyelitis began to occur in the United States and Europe. These outbreaks came as a surprise to the medical community, which viewed the disease as a rarity. The causative agent of this disease, poliomyelitis virus (later shortened to poliovirus) was identified in 1908 (Landsteiner and Popper, 1908). Research on the virus over the next 40 years provided information on antigenic types, pathogenesis, and immunity that ultimately lead to the development of two effective vaccines. A consequence of the introduction of these vaccines in the early 1960s was that investigation of the pathogenesis of poliomyelitis ceased. Instead, studies on the molecular biology, structure, and genetics of poliovirus flourished. The identification of the cell receptor for poliovirus allowed the development of transgenic mice susceptible to poliovirus, an advance that revived interest in understanding how the virus

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causes disease (Koike et al., 1991; Ren et al., 1990). Work on poliovirus pathogenesis is now done with a sense of urgency as global eradication of polio approaches.

Poliovirus is classified as an enterovirus within the *Picornaviridae*, a family that contains many human and animal pathogens. All three serotypes of poliovirus cause paralytic disease. The viral genome, a single-stranded, (+)-strand RNA approximately 7500 nucleotides in length, is enclosed in a nonenveloped capsid comprising 60 copies of four different polypeptides arranged with icosahedral symmetry. The cell receptor for all three poliovirus serotypes is CD155, a glycoprotein that is a member of the immunoglobulin superfamily of proteins (Mendelsohn et al., 1989). CD155 is composed of three extracellular immunoglobulin-like domains: a membrane-distal V-type domain that binds poliovirus, followed by two C2-type domains. Alternative splicing of mRNA leads to the synthesis of two membrane-bound isoforms, CD155α and CD155δ, and two isoforms that lack transmembrane domains and are secreted from the cell (Koike et al., 1990; Mendelsohn et al., 1989). The function of the secreted isoforms is unknown. The membrane bound isoforms are adhesion molecules: they participate in the formation of adherens junctions through interaction with nectin-3, an immunoglobulin-like protein related to CD155 (Mueller and Wimmer, 2003). CD155 is also a recognition molecule for natural killer (NK) cells; it interacts with CD226 and CD96 on NK cells to stimulate their cytotoxic activity (Bottino et al., 2003; Fuchs et al., 2004). The UL141 protein of cytomegalovirus blocks surface expression of CD155, leading to evasion of NK cell-mediated killing (Tomasec et al., 2005). CD155 and related proteins also serve as entry receptors for alphaherpesviruses (Spear, 2004).

Interaction of poliovirus with the V-type domain 1 of CD155 leads to a conformational change in the virus particle and release of the RNA genome into the cytoplasm, an event that may occur at the plasma membrane in cultured cells (Hogle, 2002). Once in the cytoplasm, the viral RNA genome is translated and the production of new infectious virions begins, a process that has been recently discussed (Mueller et al., 2005; Racaniello, 2001).

Course of poliovirus infection

Infection with poliovirus begins when the virus is ingested and multiplies in the oropharyngeal and intestinal mucosa (Fig. 1) (Bodian and Horstmann, 1965; Sabin, 1956). Virus shed in the feces of infected individuals is largely responsible for transmission of infection. From the primary sites of multiplication in the mucosa, virus drains into cervical and mesenteric lymph nodes and then to the blood, causing a transient viremia (Bodian and Horstmann, 1965). Most natural infections of humans end at this stage with a minor disease comprising nonspecific symptoms such as sore throat, fever, and malaise. Replication at extraneural sites is believed to maintain viremia beyond the first stage and increase the likelihood of virus entry into the central nervous system. Such extraneural sites might include brown fat, reticuloendothelial tissues, and muscle (Bodian, 1955; Ren and Racaniello, 1992a; Wenner and

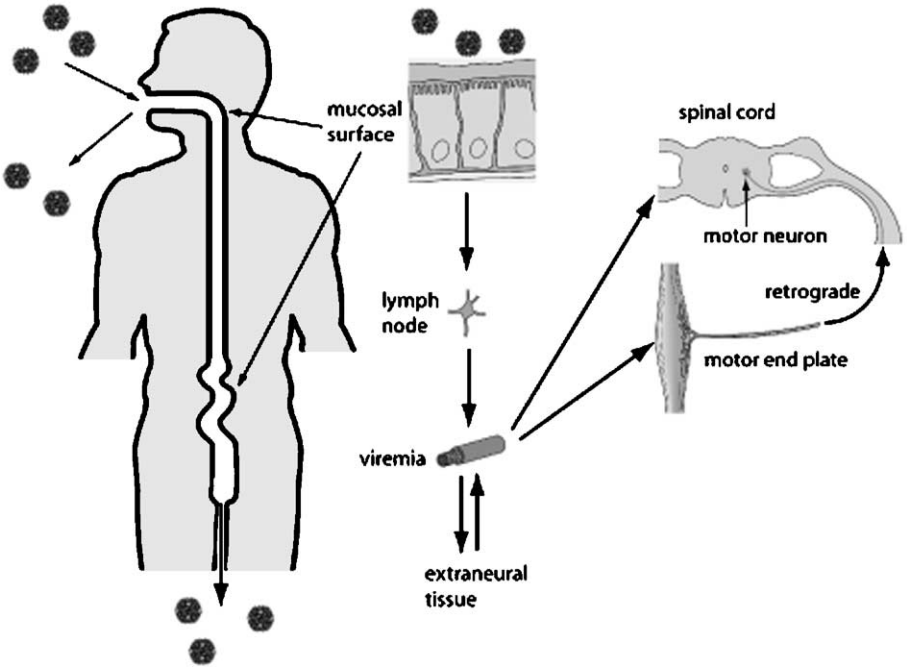

Fig. 1. Hypothetical scheme of poliovirus pathogenesis based on experimental findings in humans, monkeys, chimpanzees, and CD155 transgenic mice. Ingested virus initially replicates in the oropharyngeal and intestinal mucosa. Virus replication at these sites reaches the blood through the lymph nodes, resulting in a primary viremia. Invasion of virus into the central nervous system may occur either directly from the blood, or by retrograde axonal transport when virus enters the neuromuscular junction. It is believed that invasion of the brain or spinal cord must be preceded by viral multiplication in extraneural tissues, which leads to a sustained viremia. These extraneural tissues may include skeletal muscle and brown fat. Virus is spread most frequently by the fecal–oral route. Shedding of virus from the nasopharynx may lead to transmission of infection by the respiratory route, which occurs in developed countries with high standards of sanitation.

Kamitsuka, 1957). In 1–2% of infected individuals, the virus enters the central nervous system and replicates in motor neurons within the spinal cord, brain stem, or motor cortex. Viral replication in motor neurons within the spinal cord leads to the characteristic muscle paralysis.

Because only 1–2% of poliovirus infections lead to poliomyelitis, the neurological phase of infection can be viewed as an accidental diversion of the enteric stage. Spread of poliovirus within the population, and therefore survival of the virus, depends only on viral multiplication in the alimentary tract. Why poliovirus only rarely invades the central nervous system is not known, but a possible explanation is discussed below (see 'Tropism').

Host range

Humans are the only known natural hosts of poliovirus. Chimpanzees and old world monkeys such as rhesus, cynomolgous, and African green monkeys can be experimentally infected. The results of experiments in mice suggested that the resistance of other species to infection by poliovirus is likely due to the absence of a suitable cell receptor. Cultured mouse cells are not susceptible to poliovirus infection, but they are permissive, e.g. they produce infectious virus after transfection with viral RNA (Holland et al., 1959a, 1959b). The synthesis of CD155 in mouse L cells or in transgenic mice confers susceptibility to infection (Koike et al., 1991; Mendelsohn et al., 1989; Ren et al., 1990). Orthologs of the *CD155* gene are present in the genomes of a number of mammals, including those that are not susceptible to poliovirus infection (Ida-Hosonuma et al., 2003). The amino acid sequence of domain 1 of CD155, which contains the binding site for poliovirus, varies extensively among the nonsusceptible mammals, especially in the regions known to contact poliovirus. The absence of a poliovirus binding site on these CD155 molecules therefore explains why poliovirus infection is restricted to simians.

Certain strains of poliovirus can infect mice in the absence of human CD155. The strains P2/Lansing, P1/Lsb, and a variant of P3/Leon were selected for replication in mice by a process of adaptation involving serial passage of viruses in nonprimates (Armstrong, 1939; Li and Schaeffer, 1953). Other poliovirus strains are naturally virulent in mice (Moss and Racaniello, 1991). When mice are inoculated intracerebrally with P2/Lansing, they develop a disease with clinical, histopathological, and age-dependent features that resembles human poliomyelitis (Ford et al., 2002; Jubelt et al., 1980a, 1980b). The murine cell receptor that allows entry of these strains into mouse cells has not been identified. Substitution of a six amino acid sequence of the P1/Mahoney strain with the corresponding sequence from P2/Lansing confers mouse neurovirulence to the recombinant virus (Murray et al., 1988). This six amino acid sequence lies within capsid protein VP1, on the surface of the virion at the five-fold axis of symmetry (Lentz et al., 1997), near the binding site for CD155 (Belnap et al., 2000; He et al., 2000; Xing et al., 2000). These observations suggest that the six amino acid sequence in the capsid of P2/Lansing regulates the interaction with a mouse cell receptor, possibly by direct contact.

Entry into the host

Whether epithelial or lymphoid cells are the primary sites of poliovirus replication in the oropharyngeal and intestinal mucosa has been a matter of debate for many years. Virus has been detected in tonsillopharyngeal tissue and Peyer's patches of chimpanzees that had been orally infected with poliovirus (Bodian and Horstmann, 1965). In humans, poliovirus has been isolated from tonsillopharyngeal tissue, the wall of the ileum, and mesenteric lymph nodes (Sabin and Ward, 1941). However, removal of tonsils or adenoids does not reduce the level of poliovirus multiplication in the throats of humans (Sabin, 1956). Consequently, it is not known if poliovirus replicates in lymphoid tissues or is absorbed into lymph nodes after replication in epithelial cells.

Studies on the expression of CD155 in cells of the alimentary tract have been used to provide information on which cell types might be susceptible to infection. Human epithelial cells produce high levels of CD155 RNA, suggesting that these cells might be primary sites of poliovirus replication (Ren, 1992). In humans, CD155 protein was detected on the intestinal epithelium, M cells of Peyer's patches, and in germinal centers within the Peyer's patches (Iwasaki et al., 2002). In rhesus macaques, which are not susceptible to oral poliovirus infection, CD155 levels were reduced in follicle-associated epithelium and the protein was not present in germinal centers. These results have been interpreted to suggest that poliovirus replication in the gut depends on the presence of CD155 in follicle-associated epithelium, including M cells, and on cells of the Peyer's patches (Iwasaki et al., 2002).

CD155 transgenic mice are not susceptible to oral infection with poliovirus (Koike et al., 1991; Ren et al., 1990). CD155 protein is present at very low levels in the intestinal epithelium of these mice, and absent in the Peyer's patches (Iwasaki et al., 2002; Zhang and Racaniello, 1997). Overproduction of CD155 in the intestinal epithelium of transgenic mice by the use of a fatty acid binding protein promoter did not lead to oral susceptibility to poliovirus (Zhang and Racaniello, 1997). These findings suggest that the expression of CD155 in Peyer's patches and in M cells is important for oral susceptibility to poliovirus infection. However, these data do not exclude the possibility that poliovirus might also replicate in the intestinal epithelium. In the mouse intestine, these cells might not be permissive for poliovirus infection due to the absence of an intracellular protein required for viral replication. The production of CD155 transgenic mice that express the poliovirus receptor in Peyer's patches and M cells should resolve these questions. A line of transgenic mice (cPvr mice) has been described in which production of CD155 mRNA is under the control of the β-actin promoter (Crotty et al., 2002). The use of this promoter is expected to result in expression of transgene mRNA in all cells, although CD155 synthesis in individual cell types in the intestine was not examined. Unfortunately, cPvr mice do not develop paralytic disease after oral inoculation

with poliovirus. After intraperitoneal inoculation, low levels of poliovirus replication were detected in the intestine, but the cells harboring poliovirus were not identified. Additional work is needed to determine the block to poliovirus replication in different CD155 transgenic mouse lines.

Although CD155 transgenic mice cannot be orally infected with poliovirus, inoculation by the intranasal route leads to paralysis accompanied by virus replication in the nasal epithelium and olfactory bulb, cerebrum, brain stem, and spinal cord (Crotty et al., 2002; Nagata et al., 2004). The nasal route of infection does not appear to play a significant role in human poliomyelitis. Early hypotheses on the pathogenesis of poliomyelitis suggested that the virus entered the nose, replicated in the nasal epithelium, and spread to the brain by the olfactory pathway (Paul, 1971). Experimental findings in humans and monkeys subsequently proved that poliovirus does not invade the central nervous system by the olfactory route (Sabin and Ward, 1941). It is possible that in some cases, viral replication in the nasal mucosa leads to a viremia that allows virus to spread and eventually gain entry into the central nervous system. Although poliovirus is believed to be transmitted by fecal–oral contamination, in countries with high standards of hygiene, virus may be transmitted by the respiratory route. The source of virus for this mode of transmission is the tonsils and pharynx. Replication at these sites usually occurs after virus replication in the intestine and spread by viremia. When infection is spread by the respiratory route, it is not clear if virus replicates in the nasopharynx, or is ingested and replicates in the intestine. The study of nasal infection of CD155 transgenic mice with poliovirus may therefore have some relevance to human infections.

Spread in the host

Two routes of poliovirus entry into the central nervous system have been suggested which are not mutually exclusive: the virus enters the central nervous system from the blood, or enters a peripheral nerve and is carried to the central nervous system by axonal transport. There is ample evidence in support of both routes of entry. It has been established that viremia preceding paralytic infection is necessary for virus entry into the central nervous system. In addition, the presence of antiviral antibodies in the blood prevents invasion of the brain and spinal cord (Bodian and Horstmann, 1965). The results of experiments in CD155 transgenic mice have provided additional support for the hypothesis that virus enters the brain and spinal cord from the blood. In one study, the fate of poliovirus inoculated into the tail vein of mice was examined by pharmacokinetic analysis (Yang et al., 1997). The results indicate that poliovirus is delivered to the brain in amounts significantly greater than would be predicted from the vascular volume of that organ. Furthermore, the distribution of poliovirus in the brain of transgenic and nontransgenic mice is similar, indicating that CD155 does not play a role in delivery of circulating poliovirus to the central nervous system. The authors conclude that, in mice, polioviruses permeate the blood–brain barrier at a high rate, independent of CD155 or virus strain. The molecular mechanism of poliovirus entry by this route remains to be elucidated.

Substantial evidence for neural pathways of poliovirus dissemination has been obtained in humans and monkeys. Inoculation of poliovirus into the sciatic nerve of monkeys leads to virus spread along nerve fibers in both peripheral nerves and the spinal cord (Hurst, 1936). When monkeys are inoculated intramuscularly with poliovirus, the inoculated limb is usually the first to become paralyzed, and freezing the sciatic nerve blocks virus spread to the spinal cord (Nathanson and Bodian, 1961). In children who received incompletely inactivated poliovaccine in 1954 (the Cutter incident), a high frequency of initial paralysis was observed in the inoculated limb (Nathanson and Langmuir, 1963). Evidence for neuronal spread of poliovirus has also been obtained from experiments in CD155 transgenic mice. After intramuscular inoculation of CD155 transgenic mice, the first limb paralyzed is always the limb that is inoculated; poliovirus is first detected in the lower spinal cord, and sciatic nerve transection blocks infection of the spinal cord (Ohka et al., 1998; Ren and Racaniello, 1992b). The rate of poliovirus transport along the sciatic nerve was calculated to exceed 12 cm per day, independent of virus replication (Ohka et al., 1998). Therefore, poliovirus is carried along nerves to the spinal cord by fast retrograde axonal transport.

Skeletal muscle injury is known to be a predisposing factor for poliomyelitis, a phenomenon known as ‘provocation poliomyelitis’. For example, in Rumania, intramuscular injections have been linked to cases of vaccine-associated poliomyelitis (Sutter et al., 1992). Provocation poliomyelitis has been reproduced in CD155 transgenic mice (Gromeier and Wimmer, 1998). The mechanism by which skeletal muscle injury stimulates retrograde axonal transport of poliovirus to the spinal cord remains to be determined.

Clues about the mechanism by which poliovirus is conveyed by neural pathways are provided by the observation that the cytoplasmic domain of CD155 interacts with Tctex-1, the light chain of the retrograde motor complex dynein (Mueller et al., 2002; Ohka et al., 2004). This finding suggests the following hypothesis for axonal transport of poliovirus. At the interface of muscle and motor neuron, known as the neuromuscular junction, poliovirus binds CD155 and is taken into the neuron by endocytosis. In support of this model, CD155 has been detected at the neuromuscular junction of human muscle (Leon-Monzon et al., 1995). The endocytic vesicles containing poliovirus are linked to Tctex-1 by the cytoplasmic domain of CD155, which remains on the exterior of the vesicle. The virus-containing vesicles are transported to the motor neuron cell body, where the viral RNA is released into the cytoplasm and virus replication begins. Experimental evidence has been obtained which demonstrates that poliovirus-containing vesicles are brought to the spinal cord by axonal transport dependent upon Tctex-1 (Ohka et al., 2004). Furthermore, poliovirus appears to be transported in axonal endosomes as an infectious, 160S particle. This process differs from virus entry in HeLa cells, where interaction of poliovirus with CD155 leads to conversion of the virus to 135S particles,

which are believed to be intermediates in uncoating (Fricks and Hogle, 1990). Suppression of viral uncoating in axons may be mediated by a specific mechanism to avoid degradation of viral RNA before it can reach the cell body for translation.

Uptake of poliovirus at the neuromuscular junction also differs from the process in HeLa cells, where infection does not require dynamin and is unlikely to involve the clathrin-mediated endocytic pathway (DeTulleo and Kirchhausen, 1998).

Tropism

In the primate host, poliovirus infection is localized to specific cells and tissues, despite the presence of virus in many organs during the viremic phase (Bodian, 1955; Sabin, 1956). For many years, it was believed that poliovirus tropism was determined by the cellular receptor. This hypothesis was supported by the finding that virus binding activity in tissue homogenates correlated with susceptibility to poliovirus infection (Holland, 1961). The identification of the poliovirus receptor enabled a more extensive study of the role of receptor in poliovirus tropism. In humans, CD155 RNA and protein are expressed in a wide range of tissues, including those that are not sites of poliovirus infection (Freistadt et al., 1990; Koike et al., 1990; Mendelsohn et al., 1989). CD155 RNA and protein expression are also observed in many tissues of CD155 transgenic mice, including those where poliovirus does not replicate (Koike et al., 1994; Ren and Racaniello, 1992a). These findings lead to the conclusion that CD155 is required for susceptibility to poliovirus infection, but tropism is determined at a later stage of infection.

It has also been suggested that poliovirus tropism is determined by cell-type-specific differences in translation mediated by the viral internal ribosome entry site (IRES) (Borman et al., 1997; Gromeier et al., 1996; Ohka and Nomoto, 2001; Yanagiya et al., 2003). Cell proteins in addition to canonical translation initiation proteins have been identified that influence IRES-mediated translation (Belsham and Sonenberg, 2000). Organ-specific synthesis, localization, or modification of these proteins could lead to regulation of viral replication. In support of this hypothesis, recombinant polioviruses dependent upon the IRES of human rhinovirus type 2 or hepatitis C virus do not replicate or cause disease in the spinal cord of CD155 transgenic mice (Gromeier et al., 1996; Lu and Wimmer, 1996; Yanagiya et al., 2003). When recombinant adenoviruses were used to express bicistronic mRNAs in murine organs, the IRES of poliovirus was found to mediate translation in many organs, including those that are not sites of poliovirus replication (Kauder and Racaniello, 2004). Recombinant poliovirus dependent upon the IRES of hepatitis C virus replicated in the central nervous system, and caused paralysis in newborn CD155 transgenic mice. Previous failure to observe paralysis in mice inoculated with poliovirus dependent upon the IRES of hepatitis C virus was likely a consequence of poor replication of the recombinant virus, leading to clearance from adult mice. These results indicate that poliovirus tropism is not determined by internal ribosome entry, but at a later stage in replication.

The innate immune system, which can respond to the presence of virus within hours, can have a major influence on the outcome of infection. For example, the tropism of several viruses is regulated by alpha/beta interferon (IFNα/β) (Garcia-Sastre et al., 1998; Ryman et al., 2000). Based on this observation, the effect of the IFNα/β response on the tropism of poliovirus was determined (Ida-Hosonuma et al., 2005). Poliovirus infection of CD155 transgenic mice lacking the receptor for IFNα/β resulted in viral replication in liver, spleen, and pancreas, in addition to the central nervous system. CD155 is produced in all of these tissues, but poliovirus only replicates in the brain and spinal cord of CD155 transgenic mice that synthesize the IFNα/β receptor. In CD155 transgenic mice, poliovirus infection leads to a rapid and robust expression of IFN-stimulated genes (ISGs) (oligoadenylate synthetase, PKR, IFNα, RIG-I, MDA-5 and IRF-7) in extraneural tissues that are not normally sites of poliovirus replication. In contrast, ISG expression in the brain and spinal cord was only moderately increased after infection. These results indicate that IFNα/β functions as an important determinant of poliovirus tissue tropism in CD155 transgenic mice by protecting extraneural organs from infection.

The identification of IFNα/β as a determinant of poliovirus tissue tropism invites speculation on several aspects of poliovirus pathogenesis. As discussed earlier, poliovirus replication at the entry portal leads to a viremia, which allows virus to reach an unidentified extraneural site. Replication at this site appears to be required for virus entry into the central nervous system. We speculate that, in 99% of infections, the IFNα/β response limits poliovirus replication in extraneural tissues, thereby preventing invasion of the central nervous system. In the 1–2% of individuals in which paralytic disease occurs, the IFN response may be defective, allowing robust virus replication in nonneural sites followed by invasion into the central nervous system. It will be interesting to determine whether individuals who contract poliomyelitis have defects in innate responses to infection.

The observation that poliovirus replication in nonneural tissues of CD155 transgenic mice is limited by the IFNα/β response is surprising in light of the changes wrought by poliovirus infection on the host cell. Poliovirus infection leads to inhibition of host cap-dependent translation, DNA-dependent RNA synthesis, cellular protein secretion (including elaboration of cytokines and antigen presentation), nuclear export, and suppression of the NF-κB response (Neznanov et al., 2005). Given these effects, it is difficult to imagine that infection would be limited by IFNα/β, whose effects require many of the cellular activities inhibited by virus infection. Furthermore, poliovirus replication in cultured cells appears to be relatively resistant to IFNα (unpublished results). This apparent paradox might be a consequence of the fact that most experiments in cell culture are carried out at higher multiplicities of infection than those which occur in animals during virus infection. At lower multiplicities of infection likely to be present in tissues, the effects of virus replication on cellular processes might be significantly dampened, rendering replication sensitive to IFNα/β.

Attenuation of neurovirulence

The effort to eradicate global poliomyelitis has been conducted through the widespread use of live, attenuated poliovirus vaccines produced by Sabin. These vaccine strains infect the alimentary tract and produce immunity to infection (Sabin et al., 1954). Genetic analysis has shown that a point mutation within the IRES of each of the three poliovirus vaccine strains is a determinant of the attenuation phenotype (Evans et al., 1985; Kawamura et al., 1989; Ren et al., 1991). For example, a mutation from C to U at nucleotide 472 in the IRES of poliovirus type 3 attenuates neurovirulence in primate and murine models (Evans et al., 1985; La Monica et al., 1987; Westrop et al., 1989). This mutation has been shown to cause a translation defect in vitro and in cultured cells of neuronal origin (Gutierrez-Escolano et al., 1987; Haller et al., 1996; Svitkin et al., 1990). It has been suggested that the C472U mutation causes a translation defect that is specific to the brain and spinal cord and leads to lower viral replication in these organs (Gutierrez-Escolano et al., 1987; La Monica and Racaniello, 1989; Ohka and Nomoto, 2001). Reduced replication in the brain and spinal cord could explain the attenuated neurovirulence of the poliovirus vaccine strains.

To test this hypothesis, IRES-mediated translation was examined in mouse organs and cells. The C472U mutation was found to cause identical translation defects in neuronal and nonneuronal cells and tissues (Kauder and Racaniello, 2004). The C472U mutation therefore does not lead to attenuation of neurovirulence by specifically reducing translation in neuronal cells. Furthermore, it was found that polioviruses with the C472U mutation, which are attenuated in adult CD155 transgenic mice, replicate in newborn mice and cause paralytic disease (Kauder and Racaniello, 2004). Therefore, the C472U mutation does not eliminate viral replication in the brain. Rather, the C472U mutation might reduce viral replication sufficiently in the alimentary tract to prevent spread to the central nervous system without impairing immunogenicity of the vaccine. The reduced replication of the vaccine strains may allow the IFNα/β response to more effectively limit viral replication than it does in infections with wild type virus.

Immunization with the Sabin vaccine strains is associated with a low rate of vaccine-associated poliomyelitis, either in vaccine recipients or their immediate contacts. The rate of vaccine-associated paralysis in primary vaccines is 1 per 750,000 recipients (Nkowane et al., 1987). Vaccine-associated poliomyelitis occurs due to reversion of the mutations in the viral genome that confer the attenuation phenotype. For example, a reversion from U to C at nucleotide 472 is observed in virus isolated from cases of vaccine-associated poliomyelitis caused by Sabin type 3 (Evans et al., 1985). Such reversion events appear to occur in the gastrointestinal tract of most individuals immunized with the Sabin vaccine strains (Martinez et al., 2004). It is therefore puzzling why the Sabin vaccine causes vaccine associated disease in so few recipients. One possible explanation is that, even with selection of revertants in the alimentary tract, the replication of the Sabin strains is still sufficiently delayed in most hosts to allow containment by the immune response. Perhaps those few unfortunate individuals who contract vaccine-associated poliomyelitis have a defective IFNα/βresponse that allows revertant viruses to multiply unchecked in extraneural tissues, eventually invading the central nervous system and causing paralytic disease.

Conclusions and future perspectives

The identification of the cell receptor for poliovirus and its use in the development of transgenic mouse models for poliovirus infection has provided new impetus for understanding how poliovirus causes disease. Findings in CD155 transgenic mice have provided some answers while spawning many new questions. The identity of cells that are initially infected by poliovirus in the alimentary tract remains to be determined. To address this question, it will be necessary to determine why the intestinal tract of CD155 transgenic mice is resistant to poliovirus infection. If an orally susceptible mouse line is developed, it could be used to study the precise route of virus spread from the intestine to the central nervous system. How does the virus enter the blood? Does virus travel from the intestine to the brain stem via the vagus nerve, as does reovirus in mice? (Morrison et al., 1991). What is the mechanism of poliovirus axonal transport, what are the factors that maintain virus as 160S particles in endosome, and what is the trigger for uncoating in the neuron cell body?

The involvement of the IFNα/β response in poliovirus tropism leads to a host of questions about the role of innate responses in infection. In CD155 transgenic mice, the IFNα/β response appears to dampen virus replication in nonneural tissues due to a robust induction of ISG expression. Why is ISG expression limited in the central nervous system? What are the roles of the individual sensors of the innate response – RIG-I, MDA-5, TLR3, and TLR7/8 – in poliovirus infection, and do these roles differ in neural and nonneural organs? Poliovirus is known to inhibit many important cellular processes, including translation, transcription, and protein secretion. Why then is poliovirus replication in mice regulated by IFNα/β? Why is poliovirus infection of CD155 transgenic mice accompanied by significant inflammation, in light of the effects of infection on cellular macromolecular synthesis and protein secretion? What is the role of the innate and adaptive responses in the destruction of neurons?

The eradication of poliomyelitis has been an enormous success, and while setbacks in the program occur with regular frequency, the effort is expected to eventually succeed. Shortly after this goal is attained, it will be necessary to halt work with virulent strains of poliovirus, a step that will severely curtain research on the pathogenesis of poliomyelitis. At the moment, we know more than ever about the pathogenesis of poliomyelitis, and an enormous array of experimental tools are available to further advance the field. The dilemma is not how to answer the remaining questions, but whether there will be sufficient time to do so. Perhaps we can make sufficient inroads into the problems outlined here to establish paradigms that will help us better understand other virus diseases.

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