JOURNAL OF VIROLOGY, June 1991, p. 2767–2772
0022-538X/91/062767-06$02.00/0
Copyright © 1991, American Society for Microbiology
Vol. 65, No. 6

MINIREVIEW

Parallel Mechanisms in Neuropathogenesis of Enteric Virus Infections

LYNDA A. MORRISON[1]* AND BERNARD N. FIELDS[1,2]

Department of Microbiology and Molecular Genetics[1] and the Shipley Institute of Medicine,[2] Harvard Medical School, 260 Longwood Avenue, Boston, Massachusetts 02115

Although an enormous amount of genetic, biochemical, and structural information has been gleaned from the various applications of molecular biology to the study of enteric viruses, knowledge of the fundamental biology of these viruses and the disease processes resulting from infection by them is still incomplete. Poliovirus, the first and most devastating enteric virus pathogen of humans to be recognized, has long served as the model for studying pathogenesis of enteric virus infections. During the 1940s and 1950s epidemics of poliomyelitis acutely focused attention on poliovirus. An extensive research effort culminated in the development of effective poliovirus vaccines, but as subsequent investigation subsided significant gaps were left in our understanding of the pathogenesis of this and other enteric viruses. The biochemical basis of virion stability, the mechanics of virus entry into a host, the determinants of virus spread causing generalized infection of the host, and particularly the means of penetration by viruses into the central nervous system (CNS) all remained unresolved issues. A number of observations on the biologic processes and genetic determinants of reovirus pathogenesis, as well as advances in research on other enteric viruses, have revived (and revised) some earlier observations and interpretations of data relevant to understanding these issues. In addition to providing a framework for further study, comparisons among this group of viruses suggest that there may be common features in the pathogenesis of enteric virus infections.

VIRION STABILITY

Viruses that use the alimentary tract as the portal of entry into a host are listed in Table 1. These viruses share certain structural and biochemical features that confer on them the stability to survive the harsh environment of the gastrointestinal (GI) tract and initiate infection (reviewed in references 34 and 51). All are stable in acidic conditions, allowing them to pass through the stomach. With the exception of some coronaviruses, they are nonenveloped and are thereby unaffected by the lipid-dissociating properties of bile salts. Enteric viruses also resist inactivation by proteases present in gastric and pancreatic secretions or in fact dramatically increase in infectivity upon proteolytic cleavage (reviewed in reference 35). Some coronaviruses are activated with trypsin, possibly by cleavage of the E2 glycoprotein (48). Inhibition of proteolysis results in attenuation of rotavirus infection (56). Exposure of reovirus to chymotrypsin results in sequential digestion of the outer capsid proteins and activation of viral transcriptase activity (20). This cleavage has been demonstrated to occur in the intestinal lumen of mice (10), and inhibition of cleavage reduces viral replication in the intestine (2). Using reassortant reoviruses, the genetic determinant of protease sensitivity has been mapped to the gene encoding the outer capsid protein μ1 (40). Structural resemblance between the μ1 protein and capsid proteins of picornaviruses suggests a similar role for these proteins in maintaining virion stability and in molecular mechanisms of entry (36). Last, cleavage of the type 3 poliovirus VP1 capsid protein by host proteases can alter interactions with several types of host cells in culture (38) and may have pathogenic significance. Thus, capsid proteins of enteric viruses are important determinants of virus stability and initial infectivity in the GI tract.

ESTABLISHMENT OF INFECTION

Enteric virus infections either remain localized to the alimentary tract or become disseminated beyond it. The cell type to which virus initially binds may in part determine the ultimate course of infection and disease. Some viruses (Table 1) infect the epithelial surface of the GI tract and generally remain limited in depth to the epithelium (26, 37, 44, 58, 64). For such viruses, spread results from infection of adjacent cells, causing local symptoms of gastroenteritis and diarrhea. In contrast, reovirus and poliovirus, two viruses that spread beyond the GI tract, have been shown to selectively bind to specialized microfold cells (M cells) overlying Peyer's patches (45, 63) and to be transcytosed into lymphoid tissue (3). These and other viruses capable of disseminated infection are known to replicate in intestinal Peyer's patch and tonsillar lymphoid tissue (1, 7–9, 31, 41, 47), and their deeper penetration into the host may be facilitated by similar means of uptake in the alimentary tract. This subset of enteric viruses often causes asymptomatic or subclinical infection within the GI tract but can produce significant disease in distant tissues and organs. Certain viruses that remain localized in the intestine also adhere to M cells (12), however, indicating that binding and transcytosis by these cells is not the only determinant of dissemination beyond the GI tract.

Another factor influencing the type of infection or the extent of spread may be the propensity of a virus to bud from the apical or basolateral surfaces of cells. This phenomenon has not been studied in detail for enteric viruses; selective infection by reovirus of the basolateral surfaces of polarized epithelial cells represents the only information on this issue (39). The capacity to avoid phagocytosis and the rapidity of the immune response may also be important factors (28).

* Corresponding author.

TABLE 1. Enteric viruses and their target tissues

Family	Virus	Local intestinal infection	Systemic infection	Target tissue	CNS syndrome
Picornaviridae (human)	Poliovirus types 1–3		+	Brown fat, muscle, lymphatics, CNS	Meningitis, encephalitis, paralysis
	Coxsackievirus A1-22, A1-24, and B1-6	+	+	Intestine, brown fat, muscle, heart, CNS	Meningitis, encephalitis, paralysis
	Echovirus 1–9, 11–27, and 29–33	+	+	Intestine, brown fat, muscle, heart, skin, CNS	Meningitis, encephalitis, paralysis
	Enterovirus 69 and 71		+	CNS	Meningitis, encephalitis, paralysis
	Hepatitis A virus (enterovirus 72)		+	Liver, spleen, kidney, CNS	Encephalitis
Reoviridae	Rotavirus groups A and B	+		Intestine	
	Reovirus types 1–3	+	+[a]	All	Encephalitis[b]
Adenoviridae	Human types 40 and 41	+		Intestine	
	Human types 5, 6, and 12	+	+[c]	Intestine, CNS	Meningitis, encephalitis
	Animal	+		Intestine	
Coronaviridae	Human	+[c]		Intestine	
	Animal	+	+	Intestine, lung, CNS	Encephalitis
Caliciviridae	Norwalk group	+		Intestine	
Parvoviridae	Feline	+	+	Intestine, cerebellum, lymphatics	Ataxia
	Porcine	+	+	Intestine, reproductive tissue	

[a] In young animals, infants, and immunocompromised hosts.
[b] Serotype 3 strains.
[c] Disease association not conclusive.

VIRUS SPREAD IN THE HOST

Viruses whose infection is not confined to the GI tract spread to and localize in a variety of host tissues. The two pathways theoretically available to enteric virus for spread to distant sites in the host are the bloodstream and nerves terminating in the intestinal tissue. The two paths need not be mutually exclusive. Until recently, little was known about spread of infection via neurons in the GI tract (see below). Apparently some virus is shed into the blood (viremia) in most systemic infections with enteric viruses. Virus replicating in subepithelium and lymphoid tissue of the GI tract does not have direct access to the blood but can spread in the draining lymph to regional lymph nodes and thence to the thoracic duct and hemal circulation. The genetic basis for efficient growth in the GI tract and spread to regional lymph nodes and spleen has been identified for reovirus in studies using genetic reassortant viruses. The S1 and L2 genes conjointly influence growth, and the S1 gene (encoding the cell-attachment protein σ1) is associated with spread (9, 23).

From the bloodstream, virus must leave the vascular compartment to undergo further replication, either by transport within infected mononuclear cells or by binding the vascular endothelium of organs it will invade. Receptor populations for cell-attachment proteins present on organ endothelium may in part determine tropism of a virus. Sites of secondary replication shared among systemic enteric viruses are brown fat and skeletal muscle (7, 8, 46, 60, 61); the lymphoreticular system, myocardium, liver, and skin can also support replication (21, 27, 37, 60) (Table 1). Secondary replication may serve to amplify viremia as well.

Neurologic complications are among the most serious, though infrequent, consequences of enteric virus infections (21, 27). For most enteric viruses causing encephalitic and paralytic syndromes due to infection of neurons, scant evidence exists concerning the means by which they penetrate the respective brain and spinal cord parenchyma to produce their important clinical effects. Spread to the CNS via the bloodstream is restricted by the blood-brain barrier. This consists of a system of tight junctions between endothelial cells, a dense basement membrane, and contiguous astrocyte foot processes that form a sheath around vessels within the nervous system (19). Virus can potentially traverse this barrier to enter the neural parenchyma by infection of, or transfer across, the endothelium from the blood. Such penetration could occur in the few zones at which the endothelium is fenestrated, such as the area postrema or choroid plexus (6, 19). Infection of the choroid plexus would facilitate subsequent spread into the cerebrospinal fluid and thence to the meninges. The coxsackieviruses and echoviruses that are responsible for most cases of aseptic meningitis presumably infect the CNS by this latter route (23). Alternatively, virus could be taken up at nerve terminals in peripheral tissues around which no barrier exists.

NERVOUS SYSTEM PENETRATION

Studies of poliovirus illustrate this dilemma in determining whether a blood-borne or neural route is used by an enteric virus in spreading to the CNS (reviewed in references 8, 21, and 27). Clinically, exposure to poliovirus most often causes only silent or abortive infection. Of all apparent infections, 1 to 10% involve the CNS, resulting in meningitis and/or poliomyelitis. Histopathologic observations in human cases of poliomyelitis and in experimental infection of primates indicate that the virus has a predilection for motor neurons of the anterior horn of the spinal cord. Infection can extend to the brain, in which lesions are restricted to neurons within the CNS. Brain stem encephalitis (bulbar poliomyelitis) composes 5 to 30% of all apparent CNS infections, and multiple cranial nerve nuclei may be involved. It is unclear whether poliovirus tropism results solely from expression of viral receptor moieties on certain cell populations (62) or whether it may be influenced by accessibility of particular nerve fiber pathways (11).

Compelling data have accrued in support of neural spread, which has been postulated to occur via nerve endings in the alimentary tract (42) or in secondarily infected tissues (4). Circumstantial evidence includes the finding of virus and associated pathologic changes in the vagus and other cranial nerve nuclei, whose fibers innervate the pharynx and small intestine, in chimpanzees fed poliovirus and in patients succumbing to bulbar poliomyelitis (8, 14). Poliovirus has also been recovered from regional nerve ganglia of the alimentary tract and nodose ganglia of the vagus nerve prior to invasion of the CNS in monkeys fed poliovirus (42, 54). Lesions are not localized around blood vessels or zones of incomplete blood-brain barrier. Virus spreads via peripheral nerves to the CNS under experimental conditions. After intramuscular inoculation of monkeys, poliovirus can be detected in lumbar spinal cord and this movement can be blocked by sciatic nerve section or freezing. In addition, different sites of peripheral inoculation result in unique patterns of pathology within the CNS (18). Once within the CNS, poliovirus spreads through nerves from a single site of entry (6), following nerve tracts in monkeys and spreading to the brain after intraspinal inoculation (18, 22).

Many of these studies on neural spread employed a neurally adapted strain of poliovirus. Nonetheless, there is evidence that the experimental observations may reflect natural infection in humans. Recent tonsillectomy is a predisposing factor for development of bulbar poliomyelitis. Paralysis also has a propensity for appearing in a recently traumatized or injected limb. This provoking effect could plausibly be explained by increased access of poliovirus to nerve terminals in the limb as a direct result of injury. Alternatively, increased localization of virus in injured tissue because of inflammation-related changes in vascular permeability would provide a larger potential inoculum at local nerve terminals. Related data from the Cutter incident (33), in which numerous children were inoculated with a vaccine containing residual infectious virus, indicate that paralysis occurred more frequently in the inoculated limb.

Equally cogent arguments have been offered in favor of blood-borne spread of poliovirus to the CNS. Viremia occurs 2 to 3 days after alimentary exposure to poliovirus (7, 8, 21) and usually precedes appearance of clinical signs of nervous system involvement. The magnitude of viremia generally correlates with CNS invasiveness of poliovirus, though individual strains vary in their capacity to produce viremia (6, 17). Experimentally, characteristic cytoplasmic inclusions have been seen by electron microscopy in endothelial cells within the spinal cord of monkeys inoculated with poliovirus (5). Intravenous administration to monkeys of large doses of virus results in appearance of viral antigen in the area postrema, a region of the brain not protected by a dense blood-brain barrier (6). Last, spread to the CNS of a poliovirus strain which produces viremia and pantropic infection when inoculated intramuscularly into monkeys was not blocked by disruption of the sciatic nerve. On the basis of the assumption from these data that poliovirus can spread hematogenously to the CNS, the observation that passive or active immunization against poliovirus terminates viremia and prevents CNS infection has been considered strong evidence that viremia is directly linked to nervous system invasion.

In the long-standing controversy over route of poliovirus penetration into the CNS, recent opinion has generally favored the hypothesis of blood-borne spread (21, 25, 27, 29). The capacity of poliovirus to spread through nerves is unargued, however, and neural spread from the periphery in natural infection has remained a viable alternative awaiting proof.

Numerous parallels exist between the neuropathogenesis of reovirus serotype 3 infection in newborn mice and observations made of paralytic poliomyelitis in humans and primates. Like poliovirus, replication in lymphoid tissue of the GI tract leads to rapid spread of virus to mesenteric lymph nodes (23) and to production of viremia (52). Within 2 days of inoculation, viral titer increases in skeletal muscle and other extraneural sites (30, 46) and shortly thereafter is detected in the CNS (52). As is observed with poliovirus, titer in the blood generally correlates with severity of CNS infection among serotype 3 reovirus strains (30). Within the CNS, serotype 3 strains cause encephalitis (57). For reovirus serotype 3 strain Dearing (T3D), the genetic determinant of neuronal tropism has been identified as the S1 gene encoding the cell-attachment protein (59). The effect of virus-specific antibody on reovirus infection is also similar to that observed with poliovirus. A monoclonal antibody directed against the serotype 3 cell-attachment protein protects mice from lethal infection (55). Antibody both decreases viremia and prevents appearance of virus in the CNS after inoculation of T3D in the hindlimb footpad, without affecting replication in the hindlimb musculature (52).

However, T3D has been documented to spread by nerves and not by the bloodstream to the CNS, despite the presence of viremia (15, 50). Propensity for neural spread is a property conferred by the T3D S1 gene (50). After inoculation of T3D in the footpad, sciatic nerve transsection or treatment with colchicine to disrupt axonal transport blocks spread of virus to the lumbar spinal cord and brain (50). Evidence suggests that T3D also spreads along neural pathways within the CNS (49). Thus the observed effect of anti-reovirus antibody must be due to blockade of entry into nerves by this neurally spreading virus. Additional observations with T3D indicate that passive transfer of antibody can block nerve-to-nerve transmission of virus in mice already undergoing neuronal infection in the CNS. Such antibody treatment affords protection from lethal infection (55), decreases titer in the brain, and prevents spread of virus to the periphery (52). The demonstrated capacity of antibody to prevent penetration of, and terminate infection within, the nervous system by a neurally spreading virus calls into question the interpretation from poliovirus pathogenesis that reduction of viremia by antibody and inhibition of CNS invasion are directly linked.

The reovirus model system, in which newborn mice can be used in place of humans or primates, has provided the opportunity to examine histopathologically the critical issue of how enteric virus spreads to the CNS in the case of natural infection originating in the alimentary tract (31). The reovirus serotype 3 field isolate strain, clone 9 (T3C9), has the potential for both blood-borne and neural spread. T3C9 spreads via nerves after footpad inoculation similar to T3D (53) but generates greater viremia and is more invasive than T3D when inoculated perorally, thereby resembling many poliovirus strains. Three days after peroral inoculation of newborn mice with T3C9, viral antigen is detected in mononuclear cells of Peyer's patch lymphoid tissue by immunoperoxidase staining (31). By 3 to 4 days after inoculation neuronal cell bodies of the myenteric plexus of the ileum are also infected. Viral antigen is first detected in the CNS 4 to 5 days after inoculation. Staining is bilateral and restricted to neurons of the dorsal motor nucleus of the vagus nerve (DMNV) in the brain stem; no antigen is detected in meninges, endothelium, or CNS region with an incomplete blood-brain barrier. Infection of the DMNV is dependent on

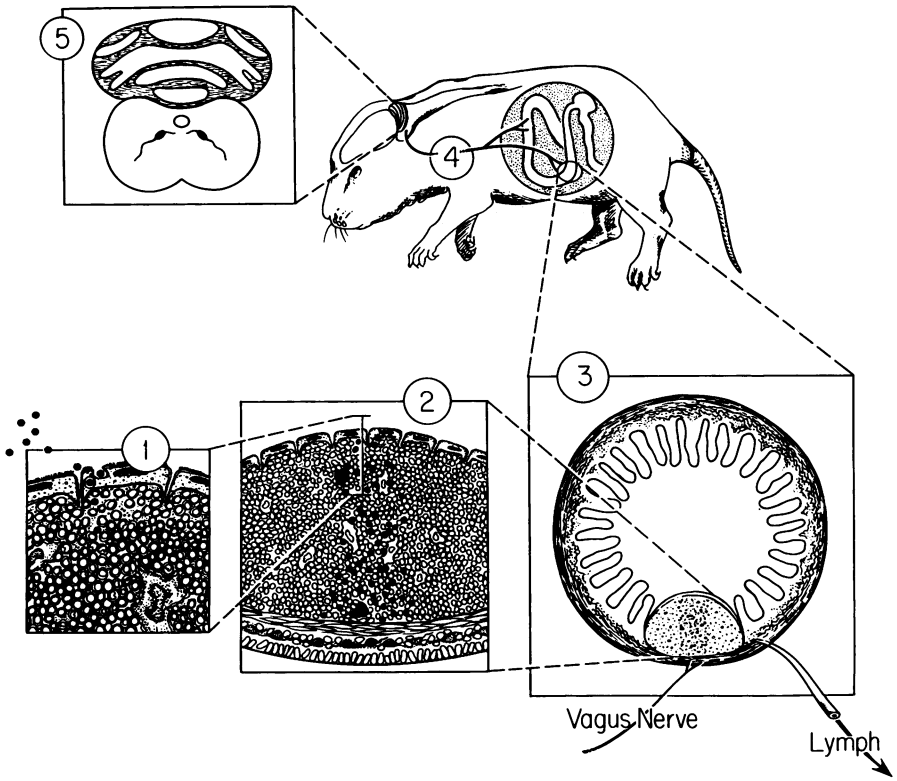

FIG. 1. Schematic representation of spread of reovirus serotype 3 from the intestinal lumen to the CNS. (1) Reovirus (and poliovirus) particles transcytosed by M cells overlying ileal Peyer's patches. (2) Reovirus replication in mononuclear cells in the Peyer's patch and in adjacent myenteric neurons between the muscle layers beneath the patch. (3) Entry from Peyer's patches into both efferent lymphatic capillaries and nerves. (4) Spread from the intestine to the CNS via vagus nerve fibers. (5) Initial infection in the CNS in neurons of the DMNV in the brain stem.

the route of inoculation and independent of level of viremia. These observations indicate that initial spread of T3C9 into the CNS occurs via the vagus nerve from the small intestine (Fig. 1). Thus, as hypothesized for poliovirus (42) and demonstrated for reovirus, enteric virus entering a host via lymphoid tissue of the alimentary tract may, under some circumstances, directly infect nerves present in the muscle wall of the tract and be transported by them into the CNS. By analogy to DMNV infection by reovirus, the development of bulbar poliomyelitis may be explained as spread of poliovirus via nerves from the intestine or tonsils to brain stem nuclei. With knowledge of the route of spread of virus from the GI tract to the CNS, the step at which antibody blocks reovirus (52) and natural poliovirus (8, 21) infection can now be more precisely defined.

Infection of neurons in the DMNV represents the initial pathway of spread into the CNS for perorally inoculated serotype 3 reovirus but does not necessarily represent the major or most important source of CNS infection. Viremia probably plays an important role by disseminating infection to muscle and organs for which a particular virus is tropic. Replication in these tissues may then permit entry of virus into local nerve terminals, promoting or reinforcing neuronal infection within the CNS (4, 24). For reovirus, there is at present no conclusive proof that virus spreads from secondarily infected muscle into motor neurons.

Viremia of sufficient magnitude under some circumstances may also permit primary or concurrent infection of the endothelium and meninges, resulting in aseptic meningitis or meningoencephalitis, respectively. The mechanisms determining whether infection of the CNS will occur, or in what manifestation, remain to be resolved. The degree of neurotropism among enteric viruses or strains of a particular enteric virus probably influences propensity for spread via nerves to the CNS. Outbreaks of enterovirus infection in which an unusually high proportion of infections result in encephalitis provide evidence of this in humans (13, 32, 60).

COMPARISON WITH OTHER ENTERIC VIRUSES

Whether observations on the pathogenesis of CNS infection by reovirus and poliovirus can be generalized to infections with other enteric viruses remains to be seen. Several observations are consistent with the possibility that parallels exist. As previously noted, several viruses have been shown to selectively adhere to M cells or to initially replicate in pharyngeal (tonsillar) and intestinal (Peyer's patch) lymphoid tissue. In CNS infections of humans, incubation periods between alimentary exposure to each enteric virus and production of CNS disease are similar. Viremia occurs within 2 to 3 days, resulting in different secondary sites of infection. Signs and symptoms appear 5 to 16 days after exposure and may be biphasic, with minor illness preceding onset of neurologic disease (43, 60). Coxsackievirus or echovirus infections of the CNS commonly cause meningitis but can produce a syndrome resembling poliomyelitis with similar pathology (60). Enterovirus 71 has been associated with polioviruslike disease and can be found in the CNS of

infected patients (13, 32) and in neurons of monkeys experimentally infected with the virus (16). The molecular determinants of pathogenesis of these viruses are largely unknown.

Hemagglutinating encephalomyelitis virus is a neurotropic coronavirus that induces vomiting and wasting disease in suckling pigs (1). In pigs inoculated oronasally, virus replicates in the lungs, tonsils, and small intestine but cannot be recovered from blood, lymph nodes, or spleen. Viral antigen can be seen in peripheral ganglia and the brain stem, suggesting neural spread from tonsils into the trigeminal nerve tract and from the intestine via the vagus nerve. These studies provide strong evidence of neural spread to the CNS for at least this highly neurotropic animal coronavirus.

Thus, other neurotropic viruses share with reovirus and poliovirus many features of pathogenesis, including mode of entry into a host, replication in secondary sites such as brown fat and skeletal muscle, incubation period to onset of illness, and patterns of encephalitic infection and pathology. Growth and virulence characteristics of individual virus strains, however, are likely to lend their individual nuances to each infection. Still enigmatic are the factors determining whether virus infection will become clinically apparent and whether it will progress to invasion of the CNS. In this regard, host factors such as age and sex, genetic susceptibility to infection, immunocompetence, and general health and nutritional status (reviewed in references 28 and 51) are likely to influence events in early stages of pathogenesis and the type and severity of disease ultimately resulting from enteric virus infections. The process by which viruses cause disease and the relationship between virus and host must be studied concurrently with identification of virion replication strategies and definition of the molecular details of attenuation and virulence. Only through such a convergence of molecular biology and pathogenesis can a thorough understanding of enteric virus infections be achieved.

ACKNOWLEDGMENTS

The authors thank Ken Tyler and Max Nibert for helpful discussion of the material presented and Greame Wilson and Ken Tyler for careful review of the manuscript.

Support for this review was received from NIAID fellowship AI08207-02, NINCDS program project grant 5 P50 NS16998, and the Shipley Institute of Medicine.

REFERENCES

1. **Andries, K., and M. B. Pensaert.** 1980. Immunofluorescence studies on the pathogenesis of hemagglutinating encephalomyelitis virus in pigs after oronasal inoculation. Am. J. Vet. Res. **41**:1372–1385.
2. **Bass, D. M., D. Bodkin, R. Dambrauskas, J. S. Trier, B. N. Fields, and J. L. Wolf.** 1990. Intraluminal proteolytic activation plays an important role in replication of type 1 reovirus in the intestines of neonatal mice. J. Virol. **64**:1830–1833.
3. **Bass, D. M., J. S. Trier, R. Dambrauskas, and J. L. Wolf.** 1988. Reovirus type 1 infection of small intestinal epithelium in suckling mice and its effect on M cells. Lab. Invest. **58**:226–235.
4. **Blinzinger, K., and A. P. Anzil.** 1974. Neural route of infection in viral diseases of the central nervous system. Lancet **ii**:1374–1375.
5. **Blinzinger, K., J. Simon, D. Magrath, and L. Boulger.** 1968. Poliovirus crystals within the endoplasmic reticulum of endothelial and mononuclear cells in the monkey spinal cord. Science **163**:1336–1337.
6. **Bodian, D.** 1954. Viremia in experimental poliomyelitis. I. General aspects of infection after intravascular inoculation with strains of high and low invasiveness. Am. J. Hyg. **60**:339–357.
7. **Bodian, D.** 1955. Emerging concepts of poliomyelitis infection. Science **122**:105–108.
8. **Bodian, D., and D. M. Horstmann.** 1965. Polioviruses, p. 430–473. *In* F. L. Horsfall, Jr., and I. Tamm (ed.), Viral and rickettsial infections of man, 4th ed. J. B. Lippincott Co., Philadelphia.
9. **Bodkin, D. K., and B. N. Fields.** 1989. Growth and survival of reovirus in intestinal tissue: role of the L2 and S1 genes. J. Virol. **63**:1188–1193.
10. **Bodkin, D. K., M. L. Nibert, and B. N. Fields.** 1989. Proteolytic digestion of reovirus in the intestinal lumen of neonatal mice. J. Virol. **63**:4676–4681.
11. **Brown, R. H., Jr., D. Johnson, M. Ogonowski, and H. L. Weiner.** 1987. Type 1 human poliovirus binds to human synaptosomes. Ann. Neurol. **21**:64–70.
12. **Buller, C. R., and R. A. Moxley.** 1988. Natural infection of porcine ileal dome M cells with rotavirus and enteric adenovirus. Vet. Pathol. **25**:516–517.
13. **Chumakov, M., et al.** 1979. Enterovirus 71 isolated from cases of epidemic poliomyelitis-like disease in Bulgaria. Arch. Virol. **60**:329–340.
14. **Faber, H.** 1955. The pathogenesis of poliomyelitis. Charles C Thomas, Springfield, Ill.
15. **Flamand, A., J.-P. Gagner, L. A. Morrison, and B. N. Fields.** 1991. Penetration of the nervous systems of suckling mice by the mammalian reoviruses. J. Virol. **65**:123–131.
16. **Hashimoto, I., and A. Hagiwara.** 1982. Studies on the pathogenesis of and propagation of enterovirus 71 in poliomyelitis-like disease in monkeys. Acta Neuropathol. **58**:125–132.
17. **Horstmann, D. M.** 1957. Poliomyelitis: problems in pathogenesis and immunization. Yale J. Biol. Med. **30**:81–100.
18. **Howe, H., and D. Bodian.** 1942. Neural mechanisms in poliomyelitis. Commonwealth Fund, New York.
19. **Johnson, R. T.** 1982. Viral infections of the nervous system. Raven Press, New York.
20. **Joklik, W. K.** 1972. Studies on the effect of chymotrypsin on reovirions. Virology **49**:700–715.
21. **Jubelt, B., and H. L. Lipton.** 1989. Enterovirus infections, p. 307–347. *In* R. R. McKendall (ed.), Viral disease. Handbook of clinical neurology, vol. 56. Elsevier Science Publishers, Amsterdam.
22. **Jubelt, B., O. Narayan, and R. T. Johnson.** 1980. Pathogenesis of human poliovirus infection in mice. II. Age-dependency of paralysis. J. Neuropathol. Exp. Neurol. **39**:149–159.
23. **Kauffman, R. S., J. L. Wolf, R. Finberg, J. S. Trier, and B. N. Fields.** 1983. The σ1 protein determines the extent of spread of reovirus from the gastrointestinal tract of mice. Virology **124**:403–410.
24. **Kristensson, K.** 1982. Implications of axoplasmic transport for the spread of virus infections in the nervous system, p. 153–158. *In* D. G. Weiss and A. Gorio (ed.), Axoplasmic transport in physiology and pathology. Springer-Verlag, Berlin.
25. **Krugman, S., and S. L. Katz.** 1981. Enteroviral infections, p. 35–56. *In* Infectious diseases of children, 7th ed. C. V. Mosby Co., St. Louis.
26. **Little, L. M., and J. A. Shadduck.** 1982. Pathogenesis of rotavirus infection in mice. Infect. Immun. **38**:755–763.
27. **Melnick, J. L.** 1990. Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses, p. 549–605. *In* B. N. Fields (ed.), Virology, 2nd ed. Raven Press, New York.
28. **Mims, C. A., and D. O. White.** 1984. Viral pathogenesis and immunology. Blackwell Scientific Publications, Oxford.
29. **Modlin, J. F.** 1990. Poliovirus, p. 1359–1367. *In* G. L. Mandell, R. G. Douglas, and J. E. Bennett (ed.), Principles and practice of infectious diseases, 3rd ed. Churchill Livingstone, New York.
30. **Morrison, L. A.,** unpublished observations.
31. **Morrison, L. A., R. L. Sidman, and B. N. Fields.** 1991. Direct spread of reovirus from the intestinal lumen to the central nervous system through vagal autonomic nerve fibers. Proc. Natl. Acad. Sci. USA **88**:3852–3856.
32. **Nagy, G., S. Takatsy, E. Kukan, I. Mihaly, and I. Domok.** 1982. Virological diagnosis of enterovirus type 71 infections: experiences gained during an epidemic of acute CNS diseases in

Hungary in 1978. Arch. Virol. **71**:217–227.
33. **Nathanson, N., and A. Langmuir.** 1963. The Cutter incident. Poliomyelitis following formaldehyde-inactivated poliovirus vaccination in the United States during the spring of 1955. Am. J. Hyg. **78**:16–81.
34. **Newman, J. F., D. J. Rowlands, and F. Brown.** 1973. A physiochemical subgrouping of the mammalian picornaviruses. J. Gen. Virol. **18**:171–180.
35. **Nibert, M. L., D. Furlong, and B. N. Fields.** 1991. Distinct forms of reoviruses and their roles during viral replication in cells and host. J. Clin. Invest., in press.
36. **Nibert, M. L., L. A. Schiff, and B. N. Fields.** 1991. Mammalian reoviruses contain a myristoylated structural protein. J. Virol. **65**:1960–1967.
37. **Pattison, J. R.** 1990. Parvoviruses. Medical and biological aspects, p. 1765–1784. *In* B. N. Fields (ed.), Virology, 2nd ed. Raven Press, New York.
38. **Roivainen, M., A. Huovilainen, and T. Hovi.** 1990. Antigenic modification of polioviruses by host proteolytic enzymes. Arch. Virol. **111**:115–125.
39. **Rubin, D. H.** 1987. Reovirus serotype 1 binds to the basolateral membrane of intestinal epithelial cells. Microb. Pathog. **3**:215–220.
40. **Rubin, D. H., and B. N. Fields.** 1980. Molecular basis of reovirus virulence: role of the M2 gene. J. Exp. Med. **152**:853–868.
41. **Rubin, D. H., M. J. Kornstein, and A. C. Anderson.** 1985. Reovirus serotype 1 intestinal infection: a novel replicative cycle with ileal disease. J. Virol. **53**:391–398.
42. **Sabin, A. B.** 1956. Pathogenesis of poliomyelitis. Reappraisal in the light of new data. Science **123**:1151–1157.
43. **Sartwell, P. E.** 1952. The incubation period of poliomyelitis. Am. J. Pub. Health **42**:1403–1408.
44. **Schreiber, D. S., N. R. Blacklow, and J. S. Trier.** 1973. The mucosal lesion of the proximal small intestine in acute infectious nonbacterial gastroenteritis. N. Engl. J. Med. **288**:1318–1323.
45. **Sicinski, P., J. Rowinski, J. B. Warchol, Z. Jarzabek, W. Gut, B. Szczygiel, K. Bielecki, and G. Koch.** 1990. Poliovirus type 1 enters the human host through intestinal M cells. Gastroenterology **98**:56–58.
46. **Stanley, N. F.** 1974. The reovirus murine models. Prog. Med. Virol. **18**:257–272.
47. **Stoddart, M. E., R. M. Gaskell, D. A. Harbour, and G. R. Pearson.** 1988. The sites of early viral replication in feline infectious peritonitis. Vet. Microbiol. **18**:259–271.
48. **Storz, J., R. Rott, and G. Kaluza.** 1981. Enhancement of plaque formation and cell fusion of an enteropathogenic coronavirus by trypsin treatment. Infect. Immun. **31**:1214–1222.
49. **Tyler, K. L., R. T. Bronson, K. B. Byers, and B. N. Fields.** 1985. Molecular basis of viral neurotropism: experimental reovirus infection. Neurology **35**:88–92.
50. **Tyler, K. L., D. A. McPhee, and B. N. Fields.** 1986. Distinct pathways of viral spread in the host determined by reovirus S1 gene segment. Science **233**:770–774.
51. **Tyler, K. L., and B. N. Fields.** 1990. Pathogenesis of viral infections, p. 191–239. *In* B. N. Fields (ed.), Virology, 2nd ed. Raven Press, New York.
52. **Tyler, K. L., H. W. Virgin IV, R. Bassel-Duby, and B. N. Fields.** 1989. Antibody inhibits defined stages in the pathogenesis of reovirus serotype 3 infection of the central nervous system. J. Exp. Med. **170**:887–900.
53. **Tyler, K. L., and H. W. Virgin IV.** Unpublished observations.
54. **Verlinde, J. D., A. Kret, and R. Wyler.** 1955. The distribution of poliomyelitis virus in cynomolgus monkeys following oral administration, tonsillectomy, and intramuscular injection of diphtheria toxoid. Arch. Ges. Virusforsch. **6**:175–182.
55. **Virgin, H. W., IV, R. Bassel-Duby, B. N. Fields, and K. L. Tyler.** 1988. Antibody protects against lethal infection with the neurally spreading reovirus type 3 (Dearing). J. Virol. **62**:4594–4604.
56. **Vonderfecht, S. L., R. L. Miskuff, S. B. Wee, S. Sato, R. R. Tidwell, J. D. Geratz, and R. H. Yolken.** 1988. Protease inhibitors suppress the *in vitro* and *in vivo* replication of rotaviruses. J. Clin. Invest. **82**:2011–2016.
57. **Walters, M. N.-I., R. A. Joske, P. J. Leak, and N. F. Stanley.** 1963. Murine infection with reovirus. I. Pathology of the acute phase. Br. J. Exp. Pathol. **44**:427–436.
58. **Wege, H., S. Siddell, and V. ter Meulen.** 1982. The biology and pathogenesis of coronaviruses. Curr. Top. Microbiol. Immunol. **99**:165–200.
59. **Weiner, H. L., M. L. Powers, and B. N. Fields.** 1980. Absolute linkage of virulence and central nervous system cell tropism of reoviruses to viral hemagglutinin. J. Infect. Dis. **141**:609–616.
60. **Weiner, L. P.** 1978. Enterovirus infections of the nervous system other than poliovirus, p. 133–143. *In* P. J. Vinken and G. W. Bruyn (ed.), Infections of the nervous system, part II. Handbook of clinical neurology, vol. 34. Elsevier/North-Holland Publishing Co., New York.
61. **Wenner, H. A., P. Kamitsuka, M. Lenahan, and I. Archetti.** 1959. The pathogenesis of poliomyelitis. Site of multiplication of poliovirus in cynomolgus monkeys after alimentary infection. Arch. Ges. Virusforsch. **9**:537–558.
62. **White, J. M., and D. R. Littman.** 1989. Viral receptors of the immunoglobin superfamily. Cell **56**:725–728.
63. **Wolf, J. L., D. Rubin, R. Finberg, R. S. Kauffman, A. H. Sharpe, J. S. Trier, and B. N. Fields.** 1981. Intestinal M cells: a pathway for entry of reovirus into the host. Science **212**:471–472.
64. **Worthington, B. B., and D. O. Graney.** 1973. Uptake of adenovirus by intestinal absorptive cells of the suckling rat. Anat. Rec. **175**:37–76.