INFECTIOUS DISEASES AND ANTIMICROBIAL AGENTS

VIRUS INFECTIONS OF THE GASTROINTESTINAL TRACT

Edited by
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Dedication to my (D.A.J.T.) wife and family.
My (A.Z.K.) mother and father, wife, children, family,
and to the memory of Jack Zakian.

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Preface

In all branches of clinical medicine, staying informed of advances made in other scientific areas is imperative. The cause of disease and its modifications, as well as diagnostic measures and the principles and basis of new treatment can be better understood this way. Gastroenterologists are well aware of this and read original articles or reviews on the physiology of the intestine, hematology, endocrinology, pharmacology, electron microscopy, and so on. However, many would think that virology is a subject they could ignore without disadvantage, and until now this was probably true. Nevertheless, in recent years virology has greatly increased our understanding of the acute diarrheal diseases, and it may yet be found that some chronic diseases are also caused by viruses. There is therefore good reason for clinicians to review the basic principles of virology and virus diagnosis and to learn about new and elegant techniques now available and to be brought up to date on what virus infections can do in the human gastrointestinal tract. This book has been put together to make this possible for the nonspecialist and specialist alike, and we are very grateful to the expert authors who have concentrated their detailed knowledge into concise and lucid prose. As often happens, valuable information has also been obtained by the study of animals, though in this case, rather than producing model illnesses in laboratory animals, it is possible to investigate diseases of farm animals produced not only by agents very similar to those that affect humans, but also under experimental conditions by agents that affect humans. Thus some research by veterinarians may answer questions we can never answer by the ethical study of humans, and also suggest where future studies of human disease may lead.

Diarrheal diseases have a striking world-wide impact. It was recently estimated that in Asia, Africa, and Latin America, during a 1 year period, there would be 3 to 5 billion cases of diarrhea and 5 to 10 million deaths. In addition, diarrhea was ranked first in the categories of disease and mortality. Diarrhea is also an important problem in the developed countries. In the Cleveland Family

study, which extended over an approximate 10 year period and included some 25,000 illnesses, infectious gastroenteritis was the second most common disease experience accounting for 16% of all illnesses.

In spite of the importance of the problem, attempts to discover the etiologic agents of a large proportion of diarrheal illnesses were unsuccessful prior to the 1970s. Major attempts to find such agents were made in the 1940s and 1950s, when volunteer studies in both the United States and Japan established that filterable agents derived from gastroenteritis outbreaks could induce diarrhea in volunteers, but in spite of this finding, attempts to culture or identify the filterable agents were uniformly unsuccessful. This lack of success was especially disappointing to virologists who, in the 1950s and 1960s, were discovering hundreds of new viruses employing the newly developed tissue culture systems; although many of these viruses were shed in the stool, not one turned out to be the long-sought virus(es) of acute gastroenteritis.

However, in the 1970s, two new groups of viruses were associated with human gastroenteritis—one was the 27 nm Norwalk virus which in 1972 was associated with an epidemic of acute gastroenteritis, and the other was the 70 nm rotavirus which in 1973 was associated with severe infantile gastroenteritis. Ironically, both groups of viruses could have been discovered many years ago, since the methods used for their detection and association with illness were available about 30 years before, when electron microscopes first came into use in the study of viruses. The Norwalk virus was first detected in stools by electron microscopy (EM) employing Norwalk convalescent serum to aggregate the particles, whereas the rotavirus was visualized initially in duodenal biopsies and later in stools. This concept of examining clinical specimens containing viruses by electron microscopy has been termed *direct virology*, a method which bypasses in vitro and in vivo systems for the study of viruses.

This approach of examining specimens directly by electron microscopy for the detection of viruses was previously described in studies involving specimens from animals. The first animal rotavirus was actually detected in 1963, when, by thin-section electron microscopic study of intestinal tissue from mice infected with EDIM (epizootic diarrhea of infant mice) virus, particles were visualized similar to those first observed in humans in 1973. Of course, it was later shown that the EDIM virus was a rotavirus and shared antigens with the human rotavirus. In addition, in 1969, rotavirus particles were visualized in stools of calves with diarrheal illness. This agent is morphologically identical to the human rotavirus visualized in stools of human infants and young children, and of course, it shares antigens with human rotavirus. A further note of historical interest were the studies reported in 1943, in which diarrhea was induced in calves with a filterable agent derived from diarrheal stools obtained from neonates involved in outbreaks of diarrhea in premature or full-term nurseries. Over 30 years later, examination by EM of calf stool obtained from an animal which

had developed diarrhea in this study revealed the presence of rotavirus particles. It is not clear whether this represented a true calf rotavirus strain or the human virus. However, over 30 years after this study human rotavirus was shown to induce diarrheal illness in the calf model. Thus, it is clear that others studying animal models had made discoveries which could have provided important leads for the study of disease in humans if their work had been recognized and pursued. In addition, examination of stools by electron microscopy had been pursued by investigators studying human hepatitis viruses in 1970; and in 1973, a year and a few months after the Norwalk virus was visualized in stools by direct virology, hepatitis A virus, another fastidious agent, was also visualized in stools by almost identical techniques.

Neither the Norwalk group of viruses nor the human rotaviruses grow efficiently in cell culture or in a convenient laboratory animal; thus, methods of direct virology have been employed in studying them. However, second and third generation tests have been developed, e.g., radioimmunoassay and immune adherence hemagglutination assay for Norwalk and enzyme-linked immunoabsorbent assay, radioimmunoassay, and a host of other methods for rotaviruses. Most of these methods do not require the in vitro propagation of these agents.

Since the first reports of human gastroenteritis viruses in the early 1970s, there has been a virtual explosion of information about agents associated with this disease. Thus, it was felt that the time was right to gather in a single source the available information on this most important subject. This book attempts to present a description of the field of viral gastroenteritis from an etiologic, epidemiologic, and physiologic perspective. In addition, in an attempt to be complete, we have included a chapter on bacteria associated with gastroenteritis, since much new information has appeared in this area also and no discussion of gastroenteritis would be complete without a presentation of the role of these agents. We have also felt it to be important to present information on the role of viruses in animal gastroenteritis. Certainly, in the field of gastroenteritis, the collaboration of those engaged in human medicine and veterinary medicine has been especially fruitful.

Hopefully, by a following edition, not only the etiology of all or almost all gastroenteritis can be elucidated, but also methods for prevention of at least a portion of the gastroenteritides—especially gastroenteritis of infants and young children—will have been developed.

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Contents

1

Some Aspects of the Classification and Basic Biology of Viruses of the Gastrointestinal Tract

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This chapter is intended to provide an introduction to those that follow. Therefore it includes a general survey of the basic biologic nature of the viruses of the types found in the human alimentary tract, of the ways in which they produce infection and disease, and of the basic principles of virus diagnostic tests. A chapter of this sort can be omitted by any who have a grounding hin human virology, but anyone who has not should read it carefully before embarking on the later, more specialized chapters. Detailed references have been omitted since there are textbooks and reviews that cover the ground fully [1,5].

Structure and Taxonomy of Viruses

A typical virus particle may be thought of as a small piece of genetic information enclosed in a proteinaceous coat to protect it from damage as it passes from one infected cell to another. It would probably be better biologic thinking to consider the virus proper as the organism replicating inside a cell, and the virus particle as the equivalent of the resistant spore form found in many more complex organisms. The genetic information is safely packaged as it passes out of a susceptible cell and is "uncoated" when it reaches a new susceptible cell. There it becomes active and replicates more copies of itself for further packaging.

Whether we start with particles or methods of replication we come to somewhat similar views of the important relationships between different viruses; we shall start by considering the structure and composition of the virus particle.

Viruses contain only one type of nucleic acid, that is, either RNA or DNA, and not both as do higher organisms like bacteria. The nucleic acid may be single- or double-stranded, whether it is RNA or DNA. The single-stranded nucleic acid may be further subdivided according to its "strandedness"—thus, enteroviruses such as poliovirus are "positive stranded" in that the RNA sequence can be, and is, directly translated through the use of the cell's ribosomes into proteins which are used for virus replication. Other viruses, such as influenza, are said to be "negative stranded" because the viral RNA is used to make a complementary copy, and it is this which can be translated to form viral peptides. A further distinction depends on whether the nucleic acid is present as one continuous strand or is segmented into two or more pieces.

All virus particles also contain proteins which surround the nucleic acid in various ways. In some viruses, such as adenoviruses, protein subunits form structural units or *capsomeres* and these in turn form a rigid icosahedral structure, the viral *capsid*; icosahedral symmetry is found in many other viruses as well, though it is particularly easy to recognize in adenoviruses. This type of outer coat may be achieved with a structure composed of a few repeating units, and in some cases it is now known how the various peptides which the virus produces are arranged within the particle. Some are found on the surface and may carry the antigens which are recognized by the immune systems of the host; others are found internally associated with the nucleic acid, and these are particularly likely to be the same in related species and become known as group antigens.

In certain viruses the nucleic acid may be seen to be arranged as a spiral structure in which, for example, in influenza viruses, the RNA is closely associated with a protein. Thus viruses of this sort are said to have helical symmetry.

If viruses bud from the surface of a cell or into the vesicles of the endoplasmic reticulum they gather an envelope of cell membrane around them. However, the peptides in this membrane are largely specified by the virus, although the lipids and glycolipids are the same as those of other normal membranes of the same type in the cell. Viruses with helical symmetry are usually enveloped, but so are some viruses with icosahedral symmetry, such as herpesviruses.

Earlier classifications of viruses referred to their size, and this in fact is still of considerable value when trying to identify an unknown virus. However, it is not a fundamental feature, and in the case of some enveloped viruses, such as coronaviruses, it can be quite variable. Ether lability was also used, and this is still a convenient way of deciding whether a virus is enveloped, since the

Table 1 Some Characteristics of Certain Viruses Found in the Human Intestinal Tract*

Family	Symmetry and size	Essential lipid ether/chloroform sensitivity	Nucleic acid	Virus
Picornaviradae	Icosahedral, 27 nm	0	ssRNA	Poliovirus types 1–3; coxsackie A, B; echoviruses
Reoviridae	Icosahedral, 80 nm	0	dsRNA, segmented	Reovirus 1–3, rotaviruses
Adenoviruses	Icosahedral, 70 nm	0	ssDNA	Higher serotypes found mainly in the gut
Parvovirus	Icosahedral, 30 nm	0	ssDNA	Uncertain whether viruses like parvoviruses found in gastroenteritis really belong to the group
Coronaviruses	Helical ?, 80–160 nm	+	ssRNA	Human enteric coronavirus not cultivated or studied in detail

*Electron micrographs of typical virus particles are shown as illustrations to other chapters.

lipid contained in the envelope can be extracted by ether and this leads to an immediate reduction in the infectivity of the virus—thus, enveloped viruses are *ether* and *chloroform labile.*

Viruses which are basically similar are also separated into serotypes if an immune serum raised against one virus has no effect on another; in a typical case the serum inactivates, or neutralizes, the virus against which it was raised but not another otherwise similar virus. There are, however, great differences in the number of serotypes which occur in different viruses. For example, there are only 2 serotypes of herpes simplex virus, whereas there are about 70 serotypes of enteroviruses. The latter are also subdivided to some extent by their

pathogenicity for laboratory animals: the polioviruses are often very neurotropic for simians, the coxsackieviruses are pathogenic for suckling mice, and the echoviruses grow readily only in tissue cultures. Some serotypes are, however, on the borderland between such groups, which are therefore convenient for practical purposes but of little fundamental taxonomic importance.

Table 1 is a summary of some of the main features of viruses which occur in the alimentary tract, or resemble those that do. Electron micrographs of many of the viruses can be found in the chapters which deal with particular organisms.

Virus Replication

There is an obvious and necessary relationship between the composition of different sorts of virus particle and the way they replicate when they enter cells, but there are also common features which arise from the fact that they are all nonmotile objects which need to come into intimate contact with the internal apparatus of the cell, without which they are unable to use or replicate the limited amount of genetic information they contain.

Viruses must first reach the cell surface, and they must be carried most of the way there passively—on air currents, in food, by peristaltic activity, and so on. Thereafter, in the vicinity of the cell, they may travel the last part of the distance by diffusion and become attached to specific receptors of various sorts found on the cell membrane. The method by which viruses enter cells is not well understood, but many seem to trigger a process akin to pinocytosis in which they become enfolded in a vacuole and drawn into the cell that way. They still have to pass the cell membrane, and this also is ill understood, but in every case the particle has to pass this barrier and become "uncoated," that is, lose its protein coat so that the nucleic acid within it is released and able to become metabolically active. The details may be different with various viruses, and are certainly more complicated than this brief description suggests. For example, it is known that some viruses, such as reoviruses, need to be digested by trypsin-like enzymes to become infective, and while enteroviruses become uncoated in the cytoplasm, adenoviruses move to the nucleus.

The replication of viral DNA resembles the processes which take place when cellular DNA replicates. From the viral DNA in the nucleus or the cytoplasm, an RNA polymerase transcribes a strand of messenger RNA which then becomes attached to polysomes in the cytoplasm and directs the production of virus peptides, which include more enzymes and the structural peptides needed to form a new virus particles. The DNA is also replicated by a DNA polymerase and incorporated into the particles of which it forms the core. Particles may form in the cytoplasm or nucleus, sometimes in crystalline arrays, and these are then released when the cell breaks down. Viruses which bud through membranes may be released from the cell surface in this process or accumulate in the cisternae of the endoplasmic reticulum.

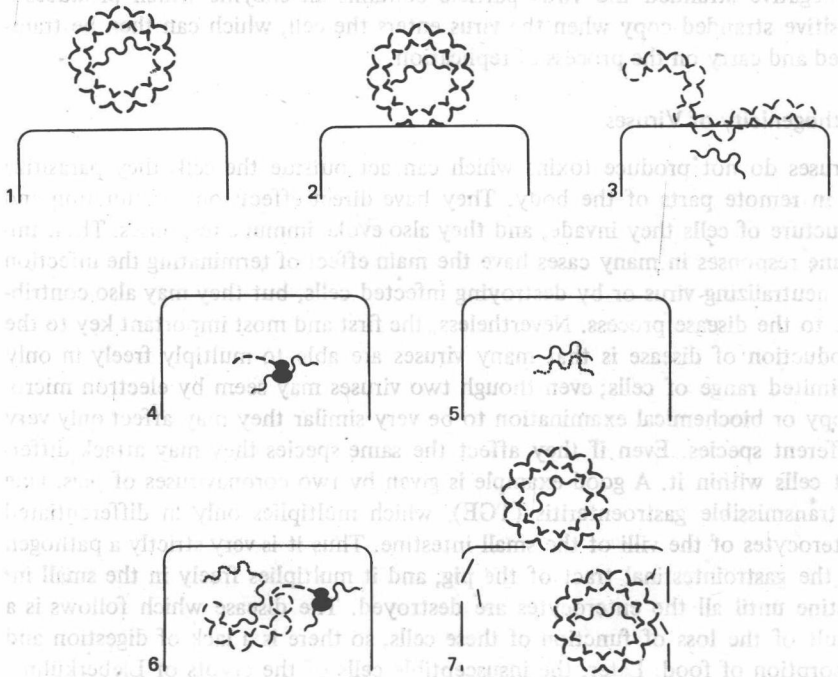

Figure 1 Scheme of replication of a simple positive-strand RNA virus. (1) Virus is adsorbed to the cell. (2) Virus particle attaches to specific receptors. (3) Virus particle becomes uncoated and RNA is released into the cell. (4) RNA attached to a ribosome directs the synthesis of virus peptide. (5) Using the virus-induced enzyme (not shown), a second "negative" strand of RNA is synthesized. (6) Using the negative strand as a template more positive RNA strands are made and incorporated into particles made of subunits. The subunits are formed by folding the peptides translated from a different section of the viral RNA. (7) The cell disintegrates, and virus particles are shed.

Viral RNA may replicate in various ways. In the simplest, the positive-stranded virus enters the cell, and is translated like an ordinary messenger RNA (Fig. 1). The peptides formed include a polymerase which produces a copy of the original RNA-negative virus strand. This in turn is copied again to produce a series of positive strands, each of which can then be inserted into a group of peptides which form a particle around it, or it may become bound to a peptide to form a nucleoprotein and this complex may lie beneath a cell membrane in which other viral proteins are inserted instead of cell proteins. The virus particle is then formed as the surface membrane bulges, then forms a bud which surrounds the nucleocapsid, and is finally nipped off. However, if the virus RNA

is negative stranded the virus particle contains an enzyme which produces a positive stranded copy when the virus enters the cell, which can then be translated and carry on the process of replication.

Pathogenicity of Viruses

Viruses do not produce toxins which can act outside the cells they parasitize or in remote parts of the body. They have direct effects on the function and structure of cells they invade, and they also evoke immune responses. These immune responses in many cases have the main effect of terminating the infection by neutralizing virus or by destroying infected cells, but they may also contribute to the disease process. Nevertheless, the first and most important key to the production of disease is that many viruses are able to multiply freely in only a limited range of cells; even though two viruses may seem by electron microscopy or biochemical examination to be very similar they may affect only very different species. Even if they affect the same species they may attack different cells within it. A good example is given by two coronaviruses of pigs. One is transmissible gastroenteritis (TGE), which multiplies only in differentiated enterocytes of the villi of the small intestine. Thus it is very strictly a pathogen of the gastrointestinal tract of the pig, and it multiplies freely in the small intestine until all the enterocytes are destroyed. The disease which follows is a result of the loss of function of these cells, so there is a lack of digestion and adsorption of food. Later, the insusceptible cells of the crypts of Lieberkühn multiply and migrate up the villus and differentiate so that, provided the animal does not die of acute dehydration, all is restored as it was before. However, a closely related virus apparently produces marked gastrointestinal symptoms by quite different mechanisms. This is the virus of vomiting and wasting disease. It infects the upper and lower respiratory tract and also the stomach and jejunum where it invades Auerbach's and Meissner's plexus. From there it spreads via peripheral nerves to the central nervous system (CNS), where it first causes marked involvement of the brain stem, spreading later to the rest of the nervous system. It seems likely that the vomiting is due to involvement of the brain stem, where the "vomiting center" is situated, and that the involvement of the plexus impairs peristalsis and so the absorption of food, giving rise to the typical wasting. It is not surprising either that, as the CNS may be widely involved, a very similar virus has been isolated from the CNS and named the hemagglutinating encephalitis virus.

In these cases the evolution of the disease can be understood as a result of the specific tropism of the viruses for particular cells. These tropisms in turn are the results of the function of the nucleic acid and proteins referred to earlier. However, there are examples showing how particular viral proteins, for instance in reoviruses [4], are involved in the virulence or tropism of the virus; thus it is