CORONAVIRUSES 9

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Viral and Other Infections of the Human Respiratory Tract. Edited by S. Myint and D. Taylor-Robinson. Published in 1996 by Chapman & Hall. ISBN 978-94-011-7932-4

9.1 INTRODUCTION AND HISTORY

The first report of a human coronavirus (HCV) was in 1965 when Tyrrell and Bynoe isolated a virus from nasal washings that had been collected 5 years earlier from a male child [168] in a boarding school [88]. This child had typical symptoms and signs of a common cold and the washing was found to be able to induce common colds in volunteers challenged intranasally. The virus, termed B814 after the number of the nasal washing, was cultivated in human embryo tracheal organ tissue but not in cell lines used at that time for growing other known aetiological agents of the common cold. The organ culture method was tried because it had already been shown that other respiratory viruses could be propagated in this manner and detected by the cessation of ciliary activity [174]. Hamre and Procknow were simultaneously working on five 'new' agents from the respiratory tract of six medical students with colds, collected in 1962 [55]. One of these, strain 229E, was adapted to grow in WI-38 cells. Almeida and Tyrrell showed that these new viruses were morphologically identical to the viruses of avian bronchitis and mouse hepatitis [4]. McIntosh and colleagues working at the National Institutes of Health in Bethesda, USA then found six morphologically related viruses that could not be adapted to cell monolayer culture but would grow in organ cultures [100]. Two of these isolates, OC (for organ culture) 38 and 43 were then adapted to grow in suckling

mouse brain. The term 'coronavirus' that described the characteristic morphology of these agents was accepted in 1968 [169].

9.2 CLASSIFICATION OF CORONAVIRUSES

Even before the term 'coronavirus' was coined, members of the family 'Coronaviridae' were well-recognized veterinary pathogens. Table 9.1 shows those members of the family that have been recognized by the International Committee for the Taxonomy of Viruses (ICTV) and their relatedness to the human viruses; this relatedness has been determined on the basis of antigenic cross-reactivity [12,51,52,58,59,68,83,86,95,109,135,160,163,178] and more recently on gene sequence data (see Structure and molecular biology). Recently it has been accepted that toroviruses should also be included in this family but, as yet, there are no known human pathogens.

There are a number of human respiratory coronaviruses described in the literature but few have been well characterized (Table 9.2). On the basis of serological cross-reactivity, however, it is possible to classify most of them [17,102,110,142]. The two main serogroups are 229E-related and OC43-related and it is the prototype viruses that will be discussed in the rest of this chapter.

Coronavirus-like particles have also been seen in the stools of humans and have become known as human enteric coronaviruses (HECVs). They are only now being characterized and at present must remain as putative members. Moreover, their role in causing disease is far from proven. In any case, as these are not respiratory pathogens, the reader is referred elsewhere for a review of these agents [28].

Other putative coronaviruses have also been described but not confirmed as coronaviruses. The Tettnang virus was isolated from the cerebrospinal fluid of a 1-year-old female with rhinitis, pharyngitis and encephalitis [116]. It was isolated originally from a suckling mouse brain culture and was shown subsequently to be a murine virus [10].

9.3 STRUCTURE AND MOLECULAR BIOLOGY

9.3.1 ORGANIZATION AND EXPRESSION OF THE HCV 229E GENOME

The hallmark of coronavirus gene expression is the production of a 3′ co-terminal set of subgenomic mRNAs in the infected cell. With the exception of the smallest mRNA, all mRNAs are structurally polycistronic but only the information encoded in the 5′ unique region of the RNA (i.e. the region absent from the next smallest RNA) is translated into protein. In the majority of cases, a single polypeptide is translated from each mRNA. The mechanism by which coronavirus subgenomic mRNAs are generated is complex and the details are

Table 9.1 Coronaviruses

Natural host	*Virus*	*Acronym*	*Relatedness to human virus*
Chicken	Avian infectious bronchitis virus	IBV	None
Cattle	Bovine coronavirus	BCV	OC43
Dog	Canine coronavirus	CCV	229E
Man	Human coronavirus 229E	HCV 229E	–
Man	Human coronavirus OC43	HCVOC43	–
Cat	Feline infectious peritonitis virus	FIPV	229E
Mouse	Murine hepatitis virus	MHV	OC43
Pig	Porcine transmissible gastroenteritis virus	TGEV	229E
Pig	Porcine haemagglutinating encephalomyelitis virus	HEV	OC43
Turkey	Turkey coronavirus	TCV	OC43

Table 9.2 Classification of human coronaviruses

Serogroup	*Prototype virus*	*Members*
A	229E	229E
		LP
		PR
		TO
		KI
		PA
		AD
		Linder
		VH
		Others
B	OC43	OC38
		OC44
		GI
		HO
		RO
Unclassified		B814
		692
		OC16
		OC37
		OC48

beyond the scope of this article. However, clearly, in common with other coronaviruses, the synthesis of HCV 229E subgenomic mRNA involves a process of discontinuous transcription and each mRNA has a leader RNA that is derived from the 5′ end of the genomic RNA [141]. It is also most likely that, as has been shown for mouse hepatitis virus (MHV) [152], HCV 229E-infected cells will contain a 5′ co-terminal set of subgenomic negative strand viral RNAs, each of which is involved in a transcriptionally active subgenomic replicative intermediate (RI) structure [151].

9.3.2 THE GENOMIC RNA OF HCV 229E

The genomic RNA of HCV 229E is a single molecule of positive-strand RNA composed of about 27 300 nucleotides and a 3′ poly-A tract of not less than 50 residues [62]. It is assumed that the 5′ end of the HCV 229E genome is linked to a 'cap' structure but this has not been demonstrated directly. At the 5′ and 3′ ends of the genome, there are non-translated regions of approximately 300 and 400 nucleotides, respectively. It can be assumed that these regions contain cis-acting elements that are needed for the replication of genomic and, possibly, subgenomic mRNAs. Within the genomic RNA, a common sequence motif, U/A CU C/A AAC (the so-called 'intergenic' region), is located immediately upstream of most HCV 229E genes. These cis-acting elements have an important role in the synthesis of coronavirus mRNAs [77, 15].

The HCV 229E genomic RNA contains eight major open reading frames (ORFs; Figure 9.1). The total number of HCV 229E gene products is, however, unknown. First, two of the ORFs, ORF 1a and ORF 1b, are polycistronic and, almost certainly, encode a number of functionally distinct proteins (see below). Second, there are many redundant ORFs located within the coding regions of larger ORFs and, at least theoretically, these could be expressed by mechanisms such as RNA editing or ribosomal

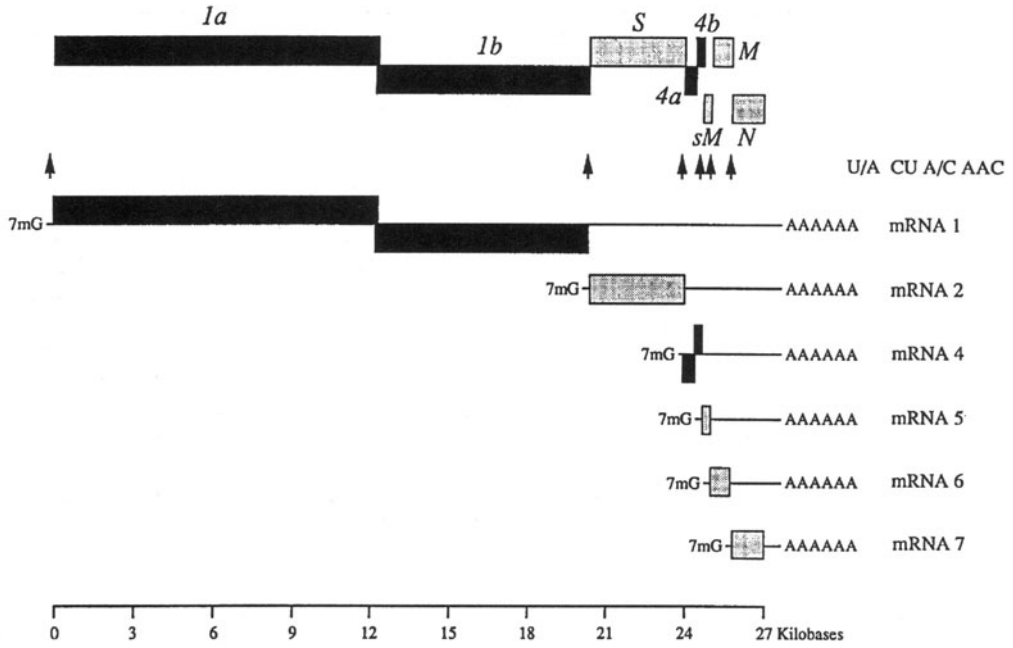


Figure 9.1 The organization and expression of the HCV 229E genome. The HCV genome is represented with eight major open reading frames (ORFs). The ORFs encoding the structural proteins (S, 5/sM, M and N) are shaded. Those encoding the non-structural proteins (polymerase/1a–1b, 4a and 4b) are solid. The position of the 'intergenic' motif (see text) and the relationship of the subgenomic mRNAs to the genomic RNA is illustrated. The transitionally active region of each mRNA is also indicated.

frameshifting. Nevertheless, it is possible to assign the HCV 229E ORFs to specific gene products. In some cases, these correspond to virus structural proteins that have been identified. In other cases, they correspond to putative proteins, the function of which can be deduced by analogy with other virus proteins. In a few cases, they correspond to putative proteins of unknown function. These assignments are listed in Table 9.3.

9.3.3 THE RNA POLYMERASE GENE (ORFS 1A AND 1B)

The HCV 229E RNA-dependent RNA polymerase gene is composed of two overlapping ORFs of 4086 and 2687 codons, respectively. These ORFs, ORF 1a and ORF 1b, encompass approximately 20 kilobases at the 5′ end of the genome. A computer-assisted analysis of the RNA polymerase genes of HCV 229E, MHV and infectious bronchitis virus (IBV) reveals a number of sequence motifs that have been associated with RNA replicative functions and protease activities in a variety of positive strand RNA viruses (Figure 9.2). These include an RNA-dependent RNA polymerase module and an NTP-dependent helicase motif located in ORF 1b and two or three putative protease domains located in ORF 1a. The protease domains belong either to the chymotrypsin/3C-like or papain-like protease groups. In addition, a motif reminiscent of a growth factor-like domain has been located in ORF 1a. The large size of the polymerase gene suggests that it may also encode many, yet unidentified, functions.

Table 9.3 The gene products of HCV 229E

Gene	*Bases*	*Codons*	*Protein*	*Molecular mass (Da)*
5′ NTR	293	–	–	–
ORF 1a	12 258	4 086	[Polymerase]	454 200
ORF 1ab	20 277	6 759	[Polymerase]	754 200
S	3522	1 174	Surface	128 600
ORF 4a	402	134	Unknown	15 300
ORF 4b	267	89	Unknown	10 200
ORF 5	234	78	[Small membrane]	9 100
M	678	226	Membrane	26 000
N	1170	390	Nucleocapsid	43 500
3′ NTR	422 $+A_n$	–	–	–

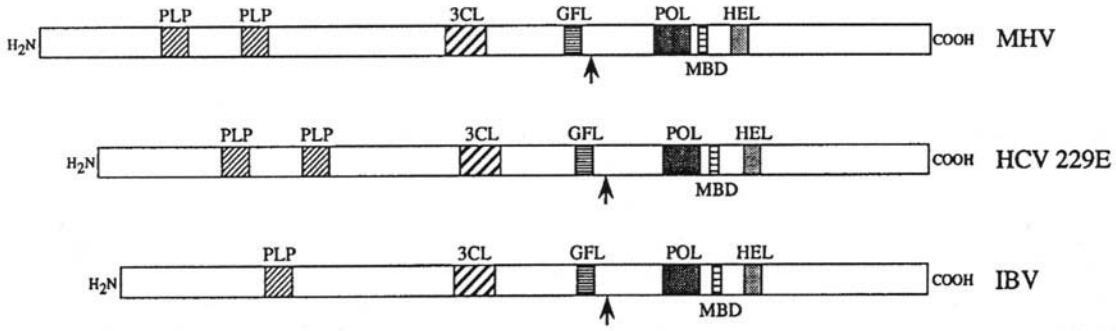


Figure 9.2 Putative functional domains in the HCV 229E polymerase. The position of putative functional domains in the RNA polymerases of HCV 229E, MHV and IBV are shown. The figure is drawn to scale, although the boundaries of the motifs cannot be defined precisely. PLP, papain-like protease; 3CL, 3C-like protease; GFL, growth-like factor/receptor; POL, polymerase module; MBD, metal binding domain; HEL, helicase (NTP binding) domain. The arrows indicate the position of the ORF 1a/1b junctions.

9.3.4 RNA POLYMERASE EXPRESSION

Ribosomal frameshifting

Expression of the proteins encoded in the polymerase gene of HCV 229E is mediated by translation of the genomic RNA, also known as mRNA 1. Translation of ORF 1a is thought to be initiated by a conventional cap dependent event at the 5′ end of the mRNA. However, translation of ORF 1b is believed to be accomplished by a (–1) ribosomal frameshifting event in the region of the mRNA that encompasses the ORF 1a/1b overlap [61]. Thus, without any further processing, the polymerase gene could encode an ORF 1a protein of 454 200 Da molecular mass and a carboxy-terminal extended ORF 1ab protein of 754 200 Da molecular mass.

The HCV 229E frameshifting event is mediated by a specific RNA structure composed of a sequence motif, the so-called slippery sequence UUUAAAC, located immediately upstream of a tertiary structure, the RNA pseudoknot (61]. For MHV and IBV, the pseudoknot is a bipartite structure, but for HCV it has been shown that an elaborated, probably a tripartite, quasi-helical pseudoknot, is required for a high frequency of frameshifting (Figure 9.3). The frameshifting event could

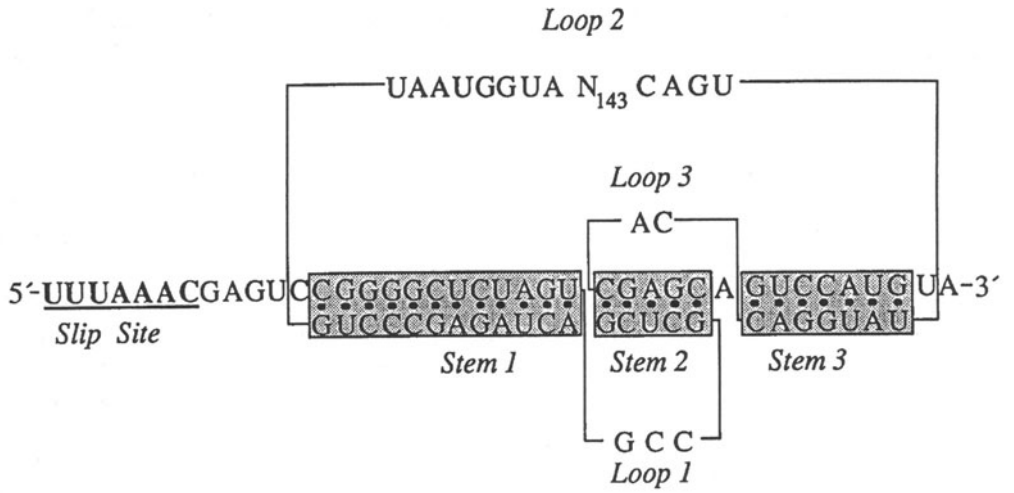


Figure 9.3 The tripartite pseudoknot of HCV 229E. A model of the HCV 229E frameshifting element, including the slippery sequence and a tripartite pseudoknot structure. A number, but not all, of the base-pairing interactions shown have been demonstrated experimentally [61].

be one of the important regulatory mechanisms of polymerase gene expression.

Proteolytic polyprotein processing

Complementation analysis of MHV temperature-sensitive (ts) mutants with an RNA minus phenotype has shown that there are at least five distinct viral functions related to coronavirus RNA synthesis [152]. Analysis of these mutants by genetic recombination allows the different functions to be located and ordered within the RNA polymerase locus [11]. The complementation frequencies of these mutants indicate intergenic, rather than intragenic recombination, so they provide strong evidence for the activity of viral encoded proteases that process the primary translation products of the polymerase gene into smaller, functional proteins. The protease motifs identified in the polymerase locus of HCV 229E (and other coronaviruses) are the most likely candidates for these activities.

Direct evidence of autoproteolytic activity encoded in a coronavirus polymerase gene has, so far, only been obtained for the first papain-like protease encoded in ORF 1a of MHV [9]. This activity has the classical features of a so-called 'leader protease' that cleaves an amino-terminal polypeptide from the primary translation product. The 3C-like protease of coronaviruses is most probably responsible for the processing of the replicative functions located in ORF 1b. There have been some preliminary attempts to analyse the proteolytic processing of the polymerase gene products of MHV [39] and similar studies on the polymerase gene of HCV 229E should follow soon.

9.3.5 THE SURFACE PROTEIN GENE (ORF S)

The HCV 229E surface glycoprotein gene encodes a polypeptide of 1173 amino acids with a molecular mass of 128 600 Da. The polypeptide has 30 potential *N*-glycosylation sites. The difference in the predicted size of the surface (S) protein and its apparent molecular weight in SDS–PAGE suggests that the majority of these sites are used. The coronavirus S protein forms the large peplomers on the surface of the virion and a number of structural features typical of coronavirus S proteins can be recognized in the HCV 229E S protein gene product [141]. These include an amino-terminal signal sequence and a carboxy-terminal membrane anchor and a cyto-

plasmic domain that displays a characteristic cluster of cysteine residues. Furthermore, the carboxyl half of the HCV 229E S protein contains two regions of hydrophobic residues that are arranged as heptad repeats. These regions are predicted to form a rigid, elongated, intramolecular coiled-coil region that would correspond to the stalk of the peplomer structure. In contrast, the amino-terminal half of the protein is thought to have a globular conformation.

In contrast to the S proteins of MHV and IBV, the HCV 229E S protein does not contain a central basic region with the motif RRXRR or RRAHR (where X is F, S, H or A). This motif has been identified as the sites at which the MHV and IBV S proteins are proteolytically cleaved. Apparently, the HCV S protein is not post-translationally cleaved.

The HCV 229E S protein has at least two major functions. First, it binds to cellular receptor(s) to initiate the infection process. A major class of receptor for HCV 229E has been identified as human aminopeptidase N [181]. Second, the S protein mediates membrane fusion. During the initial stages of the infection this function is responsible for the fusion of viral and cellular membranes at the cell surface. The structural features of the HCV 229E S protein fit remarkably well with the current view of fusogenic viral glycoproteins [94].

9.3.6 THE ORF 4A AND ORF 4B GENES

The proteins encoded by the HCV 229E ORF 4a and ORF 4b have predicted molecular masses of 15 300 and 10 200 Da, respectively. The central region of the ORF 4a gene product displays several long hydrophobic domains that may indicate a membrane-bound protein. To date these proteins have not been identified in the infected cell or in virions and they are considered provisionally as non-structural proteins of unknown function. There have been suggestions that one or both of these proteins have accessory functions and are not essential for replication in tissue culture [40,78]

9.3.7 THE SMALL MEMBRANE PROTEIN GENE (ORF 5)

On the basis of its structural similarity to the small membrane proteins of IBV and transmissible gastroenteritis virus (TGEV), the HCV 229E ORF 5 gene product is most probably a structural protein of the virus. This has not, however, been demonstrated directly. The predicted molecular mass of the HCV sM protein is 9100 Da. Besides its similarity to other coronavirus sM proteins, there is a striking structural resemblance with the M2 protein of influenza virus [98]. The influenza virus protein has an ion channel activity selective for monovalent ions and is believed to have an important role in regulating the flow of H+ ions in and out of viral and cellular compartments.

9.3.8 THE MEMBRANE PROTEIN GENE (ORF M)

The membrane glycoprotein gene of HCV 229E encodes a polypeptide of 225 amino acids with a molecular mass of 26 000 Da. The HCV 229E M protein has several features that are characteristic of coronavirus membrane proteins. First, there are three potential *N*-linked glycosylation sites, one of which is near the amino terminus. It has been shown that the HCV 229E M protein is *N*-glycosylated [87]. Second, the polypeptide displays three internal hydrophobic domains within the amino-terminal half and a relatively hydrophilic carboxy terminus. Third, the polypeptide is slightly basic with a net charge of +4 at neutral pH. These data suggest that the membrane topology of the HCV 229E M protein is very similar to that proposed by Rottier *et al.* [146] for the MHV M protein. The amino-terminal ectodomain is, however, shorter than that of MHV (16 residues compared with 25 residues).

The function of the HCV 229E M protein, in common with all coronavirus M proteins, is to bind the nucleocapsid structure to the virus

envelope during virus assembly. As the coronavirus M protein is not transported to the plasmalemma but, instead, accumulates in the Golgi apparatus, this interaction may also dictate the intracellular site of coronavirus maturation.

9.3.9 THE NUCLEOCAPSID PROTEIN GENE (ORF N)

The nucleocapsid protein gene of HCV 229E lies at the 3′ end of the genome. It encodes a polypeptide of 43 500 Da, which agrees with the apparent molecular weight of the HCV 229E N protein in SDS–PAGE [127]. In common with other coronavirus nucleocapsid proteins, the HCV 229E N protein is a serine-rich, basic protein (net charge +16 at neutral pH). The protein is most probably phosphorylated, although this has not been demonstrated directly. The distribution of basic and acidic residues is compatible with a three-domain structure, as proposed for the MHV N protein by Parker and Masters [132].

The function of the HCV 229E N protein has not been studied in any detail. However, by analogy with other coronavirus N proteins, the major function of the N protein will be to encapsidate the genomic RNA. Unfortunately, just now, there is no information on an N protein:RNA interaction that specifically leads to encapsidation.

9.3.10 THE MORPHOLOGY OF HCV 229E VIRIONS

Coronaviruses are described as roughly spherical, enveloped particles, approximately 100 nm in diameter with a characteristic 'fringe' of 20-nm long surface projections that are round or petal-shaped. The nucleocapsid structure is extended and helical. These features, and the virion proteins that comprise these structures, are represented diagrammatically for 229E virus in Figure 9.4. In this diagram, the ORF 5 gene product, the sM protein, is depicted as a structural component of the virus, although this has not yet been proved experimentally.

9.3.11 MOLECULAR BIOLOGY OF HCV OC43

OC43 virus is even more fastidious in its tissue culture requirements than 229E virus; consequently, analysis of the molecular biology of this virus has been correspondingly slow. It is clear, however, that it belongs to a haemagglutinating group which is distinguished by the presence of an additional 'haemagglutinin–esterase' (HE) gene, located between the polymerase and surface protein genes. The HE protein is a component of the virion and has receptor-binding activity, which is specific for the ligand, *N*-acetyl-9-*O*-acetylneuraminic acid as well as an acetylesterase receptor-destroying activity [158].

At the moment, however, the role of the HE protein in the replication cycle of these viruses is not exactly clear. First, it has been shown that the surface glycoprotein of bovine coronavirus

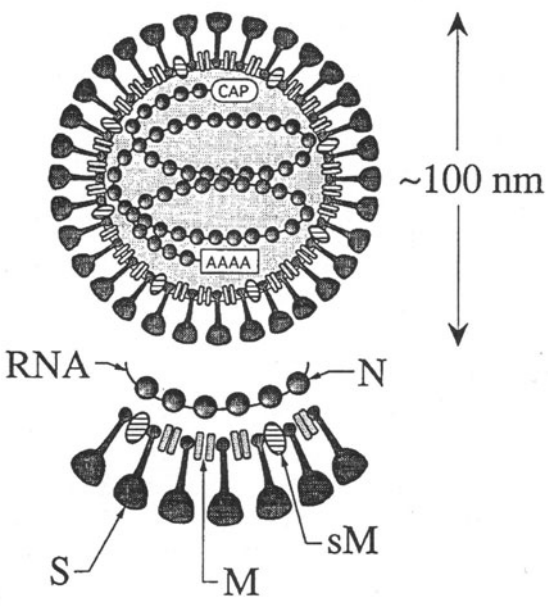


Figure 9.4 The structural components of HCV 229E, illustrated schematically. The model does not try to take account of the stoichiometry of the components nor their precise spatial relationships. RNA, genome; S, surface glycoprotein; M, membrane glycoprotein; sM, small membrane protein; N, nucleocapsid protein.

(BCV) [159] also has a strong binding affinity with *N*-acetyl-9-O-acetylneuraminic acid, i.e. it is also a haemagglutinin. Second, there is good evidence that the specific cellular receptors for MHV are members of the murine carcinoembryonic antigen (CEA) gene family [41]. And third, it has recently been shown that there is a rapid selection of HE-defective mutants during infection of mice with MHV [183]. Taken together, these data suggest that the HE protein may have an accessory function that is not obligatory. Nevertheless, this accessory function could, in certain circumstances, have an important influence on the virulence or tropism of HCV OC43 infections [184].

With the exception of the HE protein gene, the genome of HCV OC43 closely parallels that of HCV 229E regarding the RNA polymerase, surface, small membrane, membrane and nucleocapsid protein genes [155]. It differs quite significantly, however, regarding the putative non-structural genes. Thus, by analogy to MHV and BCV, there is probably an additional ORF upstream of the HE protein gene. Also, between the S and sM protein genes there is one ORF [124] that does not appear to be related to either the ORF 4a or 4b gene products of HCV 229E. In spite of these differences, it appears that the expression of the HCV OC43 genome follows the general pattern of other coronaviruses.

9.4 PHYSICOCHEMICAL PROPERTIES

Coronaviruses are susceptible to inactivation (of infectivity) by heat, acid and ultraviolet irradiation. 229E virus is more sensitive than OC43 virus to these procedures [22]. In the presence of 2% fetal calf serum, 229E virus has a 99% loss of infectivity after 10 hours at 33°C, whereas OC43 virus requires 40 hours to lose the same amount of infectivity; the same effect is produced in half of the time at 37°C. At 33°C, in the presence of 2% fetal calf serum and at pH 3.65 there is 99% loss of 229E virus infectivity after 1 hour, whereas this takes 2 hours with OC43 virus. Similarly, 229E virus is inactivated 50% more quickly by ultraviolet irradiation than OC43. Viral infectivity of 229E, however, is stable for up to 2 weeks if kept at 4°C in the presence of serum and there is only minimal loss of infectivity after 25 cycles of freeze–thawing [93], though the RNA yield from clinical material shows more rapid decay unless the virus is stored with an RNAase inhibitor (unpublished data). Chloroform (5% for 10 min) and heat (56°C for 1 hour at pH 7.0) completely abolish infectivity of 229E virus [63]. Sodium desoxycholate, sodium dodecyl sulphate, β-proprionolactone and Triton X-100 also abolish infectivity.

229E virus is rapidly inactivated by chemical disinfectants: within 1 minute after exposure to 3% hydrogen peroxide and 0.2% C4 (a quaternary ammonium compound) [120].

Virus growth is sensitive to the presence of actinomycin D, the addition of 0.1 μg/ml to L132 cells causing a 50% reduction in infectious titre of 229E virus [87].

9.5 EPIDEMIOLOGY

Most epidemiological surveys of these viruses as agents of respiratory tract illness (see Disease manifestations, p.150) have been based on serology, using either complement-fixation or haemagglutination-inhibition tests for the two prototype viruses 229E and OC43. As it is clear that serologically unrelated coronaviruses exist, the prevalence of these viruses is likely to be underestimated. Moreover, it is also certain that not all human respiratory coronaviruses have yet been adapted to tissue or organ culture. This is supported by a study based in London, UK, in which coronaviruses were isolated, by tissue or organ culture, from 18.4% of 38 patients with common colds but a further 13% of the isolates, not identifiable as other viruses, were able to induce colds in inoculated volunteers [96].

The most extensive epidemiological survey of OC43 virus infection has been the study of

the community of Tecumseh, Michigan, USA [122]. A 4-year study involved looking for serological evidence, by complement fixation or haemagglutination-inhibition, of infection with OC43 virus in 910 persons in 269 families. A mean of 17.1% of individuals showed evidence of infection with OC43 virus in any one year. There was, however, a cycling of the frequency of occurrence: OC43 virus infections occurred in most years but there were peaks of infection every 3 years. Over 80% of infections occurred despite pre-existing antibody, with infections gradually diminishing with age. These peaks took place in the winter–spring months. A study of 229E virus infections in the same community showed a mean annual rate of 7.7% infected [29]. This figure was half that recorded in a 6-year study of Chicago medical students, in which there was also marked year-to-year variation with peaks of 35% incidence [57]. Nearly 97% of 229E virus infections also occurred in the winter and spring months. The Tecumseh study showed that either 229E or OC43 virus was dominant in any one year.

This seasonal pattern was also seen in Seattle during 1975–1979, in a study in which the ELISA format was used to seek antibody rises in sequential sera from 44 adults and children in 10 families [157]. This study also showed that children had almost three times as many infections as adults.

In London, UK, the peak incidence of coronavirus infections in one study was not in winter but in summer [112]. A total of 298 sequential serum samples, collected in the years 1976–1981, were analysed for antibody rises to OC43 and 229E viruses by ELISA. Using this method, antibody could be detected for a mean of 3.5 months. Individuals appeared to get a coronavirus infection at a mean interval of 7.8 months. Another study by the same group in the same locality showed, however, that in children there was still a winter peak. In this latter study, 30% of 108 acute respiratory tract infections, in children under the age of 6 years, were found to be caused by coronaviruses as detected by ELISA. Interestingly, 30% of index cases also had lower respiratory tract symptoms of wheezy bronchitis, but none of their siblings showed evidence of such symptoms [75].

Studies of coronaviruses as causes of clinical illness have shown that they are second only to rhinoviruses as the causes of the common cold. In the USA and England, 229E and OC43 viruses are responsible for 1–30% of all clinical cases [75,84,104,131,180], with approximately an equal number of subclinical infections. Studies from around the world – Italy [30,48], Finland [143], India [118], Germany [60,148], Czech Republic [72], Japan [119,121], Romania [150] – show that coronavirus infections are just as common.

9.6 DISEASE MANIFESTATIONS

Most coronavirus infections are asymptomatic and this has been seen in seroepidemiological surveys and in human volunteer studies [15,18]. In the latter, only 10–30% of inoculated volunteers have suffered clinical effects.

9.6.1 UPPER RESPIRATORY TRACT INFECTION

The most common clinical presentation is an upper respiratory tract infection (URTI) and, in particular, the common cold. The viruses have been isolated from patients with the common cold and when inoculated intranasally into volunteers they have produced common colds [5,15,18]. There is a mean incubation period of 3 days (range 2–5 days) followed by an illness that lasts a mean of 6–7 days (range 2–18 days). The classical clinical illness is well known and consists of general malaise, headache, nasal discharge, sneezing and a mild sore throat [5]. Approximately one-tenth of individuals will also have a fever, and one-fifth will have a cough. In Table 9.4, the clinical features of rhinovirus type 2-induced common colds, those induced by coronavirus 229E, and the illness

Table 9.4 Clinical features of rhinovirus, coronavirus and influenza A respiratory tract infection

Clinical Feature	*Coronavirus 229E (%)*	*Rhinovirus 2 (%)*	*Influenza A (%)*
Fever	9–23	7–16	98
Rhinorrhoea and/or obstruction	94–100	64–100	20–30
Headache	32–85	28–50	85
General malaise	46–47	28–43	80
Sneezing	85	50	30
Sore throat	54–68	87–93	50–60
Cough	21–31	64–68	90
Hoarseness	12	57	10
Myalgia	9	21	60–75
Watery/sore eyes	29	43	60–70
Chills	18	21	90

caused by the influenza A virus are contrasted (compiled from [5,177]). It is not possible on an individual basis to distinguish rhinovirus colds from coronavirus colds, and although earlier studies suggested that there may be a different incidence of some clinical symptoms following infection with different coronaviruses, this has not been substantiated. In any case, it would not help in identifying the cause in any single patient.

9.6.2 LOWER RESPIRATORY TRACT INFECTION

Less well documented is lower respiratory tract infection associated with coronaviruses. In a seroepidemiological study, using a complement-fixation test, coronaviruses were less likely to be found in hospitalized children with lower respiratory tract infection than in controls with non-respiratory tract disease: 3.5% of 565 children in the former group had evidence of infection with OC38 or OC43 virus compared with 8.2% of 245 children in the latter group [106]. In a later study, however, of 417 hospitalized children under 18 months of age with lower respiratory tract disease, there was serological evidence of either 229E or OC43 virus infection in 8.2% [105]. The incidence of coronavirus infection was higher than that of other respiratory viruses, except parainfluenza virus type 3 and respiratory syncytial (RS) virus. It is unlikely that there is direct infection of the lower respiratory tract and that any association is through secondary phenomena. Though these secondary phenomena are yet to be defined, a link between coronavirus infection of the upper respiratory tract and wheezing attacks has been shown in several studies over the last 20 years [75,76,105,112]. The basis of this is unclear although a correlation between severity of cold symptoms and IgE levels in nasal secretions was shown in one study [25]; there did not, however, appear to be a correlation with systemic atopy, as assessed by pin-prick to common allergens, or with mediator (leukotriene C4, leukotriene B4 and histamine) release in nasal secretions. In clinical studies, asthmatic children appear to be at particular risk of virus-induced wheezing. In this situation up to 30% of acute wheezing episodes may be due to coronavirus infection. Increased airways resistance has been shown to occur in the upper respiratory tract in non-atopic individuals with colds [2,14] and asthmatic individuals are likely to be at greater risk of this being generalized in the respiratory tract.

9.6.3 NON-RESPIRATORY TRACT INFECTION

Non-respiratory tract infection has also been described with coronaviruses and includes multiple sclerosis, pancreatitis, thyroiditis,

pericarditis, nephropathy and infectious mononucleosis when seroconversion has been sought [7,8,145]. The association with multiple sclerosis has been of particular interest since coronavirus-like particles were seen in the post-mortem brain of a patient who died with the disease [164]. Subsequently, OC43-related coronaviruses, SD and SK, were isolated from the brain material of two multiple sclerosis patients [23]. However, it is likely that these isolates were murine coronaviruses present in the mice used for cultivation [44,45,179], although a lack of neutralization, in a plaque assay, of SD or SK with OC43 antiserum was shown in one study [46]. Seroepidemiological studies in which attempts have been made to ascertain an association with multiple sclerosis have been conflicting in that either an association has not been found or when seen, it has been to either OC43 or 229E virus [70,97,114, 148]. Gene detection has failed to detect OC43 virus either by standard probes [161] or polymerase chain reaction (PCR) but has suggested a neurotropism for 229E virus [78]. There is also evidence of coronavirus transcription in the brains of patients with multiple sclerosis [162]. In addition, a five amino-acid motif on mRNA 4 of 229E virus is shared with myelin basic protein [78]. It is clear that further research is needed in this area.

The role of coronaviruses in causing diseases outside the respiratory tract has been doubted mainly because there has been no evidence that the virus spreads from the nasal mucosa. In support of this has been the finding that human serum contains one or more natural non-antibody inhibitors to coronavirus OC43 [36–38]. It is possible that 229E virus evades such mechanisms, however, as it is capable of replicating in macrophages which may not only impair the immune response but may also be a route of spread of the virus [134]. Recently it has also been possible, using the PCR, to show that there is a short viraemic phase with 229E virus in at least some experimentally inoculated volunteers (unpublished data). Allied to this, the identification of the 229E virus receptor as a metalloprotease [181] which is found on cells of many tissue types would also suggest greater possibilities for disease causation than just the common cold.

Although it is likely to be due to secondary phenomena, coronaviruses, in common with influenza viruses and rhinoviruses, have profound and occasionally prolonged effects on the psychology and psychomotor performance of individuals [20,33,166]. It is these effects which may provide an even more important rationale for the development of therapeutic and preventive measures to coronavirus infection as the economic consequences are great.

9.7 PATHOGENESIS AND IMMUNE RESPONSE

Not only the virus but also the host plays an important role in the pathogenesis of common colds. Everyone knows someone who claims that they never suffer from a cold. One factor appears to be the psychological state of the individual [20,33,166], though environmental [90] and genetic factors also play a role; susceptibility to 229E virus infection has been shown to reside on the q11-qter region of human chromosome 15 in a study using murine–human hybrid cell lines [147].

Little is known about the detail of pathogenic mechanisms in human coronavirus infection, principally because man is the only model of infection. Even the predominant mode of transmission is uncertain. Infection can be induced experimentally by direct inoculation of virus into the nose but this is unlikely to be the natural route. By analogy with rhinoviruses it is likely to be either by aerosols or fomites [43] with the former a more likely explanation for the frequent simultaneous transmission that is seen to those exposed. In support of this is the finding that 229E virus survives well in an atmosphere of high humidity and low temperature [74].

Once in the nose the virus is thought to enter by a specific receptor, aminopeptidase N

(CD13) in the case of 229E virus and a sialic acid-containing receptor in the case of OC43 virus (see Structure and molecular biology, p.142). It is suggested by ultrastructural studies that it is ciliated cells, and not goblet cells, that are infected [1]. *In vitro*, 229E virus appears to be randomly distributed on the surface of cells at 4°C but redistributes using an energy-dependent mechanism on heating to 33°C [133]. Replication then takes place over the next 8–24 hours; in MRC-c and WI-38 cells, 229E virus can be found in rough endoplasmic reticulum 12 hours after infection, and in cytoplasmic vesicles and extracellulary 24–36 hours after infection [13,108]. Replication is optimal at 32–33°C, the temperature in the superficial layers of the nasal mucosa. Virus budding then takes place from Golgi apparatus with little loss of cells. Cilia appear to be withdrawn into the cell and it may be this which is associated with rhinorrhoea [1] although increased plasma exudation of proteins such as fibrinogen, which may be histamine-responsive must also play a role [3,54]. Serum antibody levels rise after about a week but it is not clear whether it is this response or cell-mediated mechanisms which clear infection [26]. Certainly there is some, inverse, correlation of the severity and likelihood of disease with pre-existing serum antibody but the mere presence of such antibody is not protective [24]. Serum antibody levels peak about 2 weeks after infection and decline to low or undetectable levels at 12–18 months. The serum antibody response is directed mainly against the surface protein but there is also an, albeit lesser, antibody response directed against the nucleoprotein and membrane proteins [111,154].

Interferon, specific nasal secretory IgA and total nasal secretory protein appear to play a role in protection from infection [172]. The last component may have natural antiviral properties [117]. 229E virus has been shown to be a good inducer of interferon in natural leucocyte cultures derived from healthy children [138]. Other non-specific factors, such as a rise in local temperature that occurs during nasal blockage, may be important not in resistance but in aborting an infection as they may induce the production of heat-shock proteins, activate lymphocytes and inhibit viral replication.

The cell-mediated immune response has not been investigated in natural infections but sera from volunteers has been shown to possess antibody that can elicit antibody-dependent cellular toxicity *in vitro* [69].

Whatever the relative importance of different arms of the immune response in clearing coronavirus infection, re-infections are common and can occur within a year to the same serotype or within 2 months to a heterologous serotype [29,96,157].

9.8 DIAGNOSIS

Because of the trivial and temporary nature of common colds, the detection of coronavirus infections has not been attempted in routine diagnostic laboratories. This situation is unlikely to change unless antiviral therapy becomes available. Although most techniques available to the diagnostic virologist have been used to detect human respiratory coronaviruses, even in research laboratories the range of tests employed by any one centre tends to be limited. A synopsis of the range of techniques used is presented below, but the reader is referred elsewhere for details of methods [128].

Organ culture

The method, or modifications of it, developed by Tyrrell and Bynoe [168] to isolate B819 virus is still used by some laboratories but is hampered by the difficulty of obtaining human embryonic tracheal tissue. It remains, however, the best method available for primary isolation of the broadest range of respiratory coronaviruses.

Tracheal tissue is taken from 14 to 24-week-old embryos and planted in sterile plastic Petri dishes containing 199 medium. The tissue is immersed with the cilia uppermost. Virus can

be inoculated onto the cilia and the tissues are then incubated at 33°C for up to 10 days. Viral replication is indicated by cessation of ciliary activity and confirmed by interference with another virus (echovirus, parainfluenza or Sendai). Electron microscopy was used originally to confirm that isolates as coronavirus but other tests such as neutralization are now employed.

Trachea obtained from 5 to 9-month-old fetuses has also been shown to support the growth of coronaviruses, and different media recipes can also be used [55].

Mouse brain culture

OC38 and OC43 viruses (but not B814, OC16, OC37, OC44 or OC48) have been adapted to grow in suckling mouse brains [55,101,103, 170]. The mice can be inoculated intracerebrally or via the peritoneum, with encephalitis occurring some days later. In the initial description by McIntosh and colleagues, CD-1 Swiss mice were used, with encephalitis occurring 11–15 days after inoculation with virus that had been passaged several times in organ culture. After the fourth passage, the time to illness was reduced to 40–60 hours. Evidence of infection was not found in other organs (liver, heart or lungs). Virus can be prepared from brain suspensions by clarification through low-speed centrifugation and then adsorption to and elution from group O erythrocytes or by more elaborate ultracentrifugation-based procedures [82]. Virus may be visualized by electron microscopy. Although this is not used for primary isolation of virus, this method is still a commonly used means of preparing OC43 antigen for serological assays.

Cell culture

Cell cultures have proved unreliable for primary isolation of all human respiratory coronaviruses but certain strains have been adapted to growth in them. The 229E virus and related strains grow well in a continuous heteroploid cell line termed C16 because they were the sixteenth clone of MRC-c cells that was selected [137]. The original description of these cells from the MRC Common Cold Unit in Salisbury, indicated that morphologically they were a mixture of fibroblastic and epithelioid cells. The former constituted three-quarters of the cell population. These cells were contaminated with organisms detected by Hoechst stain 33258, presumably *Mycoplasma* spp. Interestingly, attempts to remove this contamination with agents, such as ciprofloxacin, have resulted in a poorer yield of virus from the cell line (unpublished data).

C16 would, arguably, be the cell line of choice for the isolation of 229E virus but frequent passage of these cells results in an increasing proportion of the epithelioid content and a consequent reduction in the ability of the cell line to sustain detectable replication of virus. Many laboratories have continued to utilize a cell line that was originally used in the work of Hamre and Procknow [55]: a human diploid cell strain from the Wistar Institute, so-called WI-38 cells. Although 229E virus was readily adapted to this cell line, primary isolation was in human kidney cells and the authors noted that WI-38 cells may not be ideal for primary isolation.

Apart from C16 and WI-38 cells, many other cell lines and strains have been used to grow individual virus strains [16,19,21,31,32,56,79, 153,154,171]. These are summarized in Table 9.5. These viruses do not grow with easily recognizable cytopathic effects in cell types commonly used for the isolation of other respiratory viruses, such as HEp-2, rhesus monkey kidney or MRC-5, which makes routine identification difficult. It is, moreover, clear that the ideal cell line is not yet available and other methods of diagnosis have been applied.

Haemadsorption

OC43 virus, once adapted to BSC-1 cells, can be detected in cell culture by an ability to adsorb rat and mouse erythrocytes.

Table 9.5 Cell lines and strains used for the cultivation of human coronaviruses

Cell type	*Virus*	*Primary isolation or adaptation*
Human embryonic kidney (HEK)	229E	Primary
C16 (see text)	229E	Adaptation ⩾ primary
Human embryonic lung fibroblast, WI-38	229E	Adaptation ⩾ primary
Human embryonic lung fibroblast, MRC-c	229E and OC43	Adaptation > primary (OC43, adaptation only)
Human embryonic lung epithelium, L132	229E and OC43	Adaptation > primary
Human embryonic intestinal fibroblast, MA177	229E	Primary isolation of some strains
Human type II pneumocytes	229E	Primary > adaptation
Human fetal tonsil fibroblast (FT)	229E and OC43	Adaptation
Human embryonic rhabdomyosarcoma (RD)	229E and OC43	Adaptation
Primary monkey kidney (PMK)	OC43	Adaptation
Rhesus monkey kidney epithelium (LLC-MK2)	OC43	Adaptation
Continuous green monkey kidney epithelioid (BSC-1)	OC43	Adaptation

Adsorption of human, monkey and guinea pig cells occurs poorly, if at all [80].

Electron microscopy and immune electron microscopy

Electron microscopy of nasal washings is impractical as the virus load is usually below the level of sensitivity of standard methods. It has been used, however, to detect virus in tissue sections, such as in mouse brain culture of OC43 virus. It is also the means by which HECVs have been detected [27]. Negative staining with tungsten has usually been the method of choice, but molybdenum and uranium salts have also been used. The particles appear larger if uranium salts are used in place of tungsten [35].

An attempt to enhance electron microscopy by utilizing antibody concentration of cultured virus has been used successfully to detect 692 virus in washings from an adult with upper respiratory tract infection [81]. Nasal washings were passaged through both cell culture and tracheal organ culture and the resultant supernatant fluid was then incubated with convalescent serum from the same patient. After centrifugation the pellet was examined on a Formvar–carbon-coated grid. Aggregates of virus were clearly discernible. Supernatant fluid that had been incubated with phosphate-buffered saline, instead of convalescent serum, was also examined but virus particles were not seen.

Immunofluorescence

An immunofluorescence method has been developed and applied to the detection of 229E and OC43 viruses in nasopharyngeal secretions and washings [107]. Sera were raised against mouse brain-derived OC43 virus and cell culture-grown 229E virus in rabbits and used in an indirect fluorescence assay. This test was able to detect homologous coronavirus antigens in nasal washings from infected volunteers, though cross-reactivity was noted with the 229E virus antiserum in washings from volunteers who had been inoculated with OC43 and OC44 viruses. No nasopharyngeal aspirates from 106 children who were hospitalized with respiratory tract infections had detectable coronavirus antigen by this method. It is difficult to ascertain whether this was due to a lack of sensitivity of this system as paired sera collected from 66

children during the study period did not show evidence of coronavirus infection.

Enzyme-linked immunoassay (ELISA)

An ELISA method based on purified 229E virus and HECV CV-Paris (which has cross-reactivity with OC43 virus) has been used to diagnose infections in children [110,111]. The ELISA method is a modification of that described for antibody detection using rabbit antisera (see below). In a study of 30 children aged 6 months to 6 years, 159 samples were collected: 111 nose swabs, 11 throat swabs and 55 nasopharyngeal aspirates. Some 34.2% of the nose swabs were positive for either 229E or OC43 virus, but only 18.2% of the throat swabs and nasopharyngeal aspirates. No comparison with serology was attempted but the positivity rate would suggest that this ELISA was a sensitive test.

Time-resolved fluoroimmunoassay

Using monoclonal antibodies raised in Balb/c mice, a time-resolved immunoassay has been developed to detect OC43 and 229E antigens. When compared with monoclonal- and polyclonal-based ELISA assays, it was a more sensitive procedure than either: 100% of known positive nasopharyngeal aspirates were identified with the assay whereas only 69% of 229E infections and 90% of OC43 infections were diagnosed by the monoclonal-based ELISA. The sensitivity of the assay was determined as 0.308 ng virus for 229E and 0.098 ng virus for OC43 [65].

Nucleic acid hybridization

A gene detection method for human coronaviruses was first applied to 229E virus using Northern hybridization [126,127]. A cDNA that encoded the entire nucleocapsid gene for 229E was ligated into a Riboprobe (RPromega) vector, pGEM-1, from which ^{32}P-labelled full-length transcripts could be generated. These transcripts could be made as sense or antisense depending on whether an SP6 or T7 promoter was used. This method has been applied to the detection of 229E virus in nasal washings from inoculated volunteers and has been shown to be at least as sensitive as culture, with the advantage of a diagnostic result being available within 48 hours. An interesting feature was that the probe was able to detect virus for longer than cell culture in sequential samples from the volunteers. This probe method will not detect OC43 virus and attempts to remove the radioactive labelling by incorporating biotin or digoxigenin into transcripts have led to significant loss of sensitivity (unpublished data).

Reverse transcription–polymerase chain reaction (RT–PCR)

With the advent of gene and probe amplification strategies it was to be expected that these methods would be seen as advantageous for these difficult to cultivate viruses. Gene amplification methods based on 'nested' priming have been shown to be a sensitive and specific means of detecting both 229E and OC43 viruses [129]. Serotype-specific 'nested' primers were designed from the known sequences of the nucleocapsid genes of 229E and OC43 viruses. The inner primers were, in particular, chosen to produce a small fragment of about 100 base pairs for maximum sensitivity. Ribonucleic acid (RNA) is extracted using an acid–phenol–guanidium isothiocyanate procedure followed by reverse transcription using murine Moloney leukaemia virus RT. Two 20-cycle amplification steps are then used with the outer and inner sets of primers, respectively. The sensitivity of the assay appears to be much greater than that of cell culture or probe and each primer pair appears to be either 229E or OC43 virus-specific. The use of this method has greatly enhanced the diagnostic yield of coronaviruses in clinical material from asthmatic children [76]: the assay

is at least as sensitive as a combination of culture and serology for diagnosing infection and is more specific. RT–PCR is likely to become the method of choice for direct virus detection.

Serological methods

Because of the lack of reliable detection methods before the development of those using gene detection, most epidemiological studies have been based on serological assays to determine evidence of coronavirus infection. The most widely used and sensitive format is the ELISA. The assay was first described for virus strain 229E [92] but has since been adapted for detection of antibodies to OC43 virus [91,113,156]. The 229E virus assay uses antigen that is grown in cell monolayers and then clarified. The OC43 virus test uses mouse brain-derived antigen. Rabbit antisera have been used for both 229E and OC43 tests. The specificities of the assays are similar to those of counter-immunoelectrophoresis, neutralization and complement-fixation, but sensitivity is over 1000-fold greater. In volunteer studies, the 229E virus assay has shown a close correlation between clinical illness and virus shedding. It has been the principal method for determining the occurrence and frequency of coronavirus infections in serological surveys but recent data suggest that some false-positive and false-negative reactions occur [129]. The 229E virus assay also detects antibody rises to some 229E-like viruses (PR, KI and TO). There also appears to be cross-reactivity of 229E virus with MHV-3 in the specified format, due to bilateral cross-reactions between the S protein of MHV-3 and the N protein of 229E virus [91]. The use of recombinant S protein as antigen may overcome some of these problems [139].

OC43 virus will also agglutinate chicken, mouse and rat erythrocytes at 4°C, 20°C and 37°C [82]. Human and monkey cells can be agglutinated at 4°C but not at the other two temperatures. This phenomenon is abolished by trypsin or Tween-80/ether treatment of virus. A haemagglutination-inhibition test based on this phenomenon was specific and did not detect other haemagglutinating viruses such as influenza virus.

Other serological test formats have also been used: indirect haemagglutination [85] and immune-adherence haemagglutination [47] for 229E virus antibody; rapid microneutralization [49] and plaque-reduction [50] for OC43 virus antibody; and immunofluorescence [123], complement-fixation [71] and single radial haemolysis [64,144] for both 229E and OC43 antibody. These test formats have been superseded by the ELISA.

9.9 MANAGEMENT AND THERAPY

The management of common colds due to coronaviruses is the same as that for other causes, essentially aimed at symptomatic relief [99]. Such general remedies are summarized in Table 9.6. The use of local hyperthermia is, particularly, interesting as it has undergone more rigorous scientific evaluation than other measures [130,173,175,182]. An apparatus was developed to deliver hot, humid air into the nares at a temperature of 43°C and a rate of 4 l/min. It was hoped that this would have an antiviral effect in that this temperature should be non-permissive for the replication of either rhinoviruses or coronaviruses; moreover, there might be stimulation of lymphocyte activity or heat-shock proteins. Initial controlled studies were promising, with 55% of volunteers treated daily for 20 minutes being asymptomatic, compared with only 10% of those who had air at 30°C [175]. The existence of an antiviral effect was, however, not shown. The use of intranasal nedocromil, a mediator blocker, on coronavirus colds has, similarly, been tried with some clinical benefit but without any effect on virus shedding [176].

Specific therapy has, however, been tried with intranasal recombinant human interferon-α. In a double-blind placebo-controlled study

Table 9.6 Popular remedies for the common cold

Remedy	*Possible effect(s)*	*Comments*
Aspirin*	Antipyretic	Increases viral shedding in experimental rhinovirus colds.
	Analgesic	
		Diminishes efficiency of mucociliary mechanisms
Paracetamol*	Antipyretic	
	Analgesic	
Caffeine*	Analgesic	Alters mood and sleep
	Anti-sedative	Raises blood pressure
		Biochemical side effects
		Side effects outweigh any theoretical benefit
Anticholinergic compounds* (atropine, scopolamine)	Reduce nasal secretions	Contraindicated in glaucoma
		Multiple side effects (urinary retention, constipation, confusion etc.)
		No evidence of efficacy
Alcohol	Sedative	
Antihistamines* (chlorpheniramine, brompheniramine)	Reduce secretions	Probable 'drying' effect due to anticholinergic activity rather than as antihistamines
	Sedative	
Sympathomimetics* (pseudoephredine, ephredine, phenylephrine, phenylpropamine)	Reduce secretions	Have been shown to reduce nasal airways resistance and provide symptomatic relief if applied locally. Rebound hyperaemia, however, occurs
Antitussives* (dextromethorphan)	Reduce cough	Centrally acting
Expectorants* (guaifenesin, ammonium chloride, ipecac, terpin hydrate)	Reduce cough	No evidence of efficacy
Menthol and other inhalations	Centrally acting	Provides symptomatic relief from congestion
		Does not change airways resistance
Garlic	Reduces risk of contact	No large case-controlled studies
	Has antimicrobial properties	
Vitamin C	Inhibits prostaglandin synthesis[140]	Marginal benefit shown in studies so far[53]
	Antiviral (bacteriophages)[125]	Aspirin potentiates uptake of ascorbic acid in common colds
	Antihistamine	
	Immunomodulation[165]	
	Reverses decrease in leukocyte ascorbic acid levels[73]	
Steam inhalation	Loosens tenacious secretions	Symptomatic relief only
Zinc lozenges	Inhibits post-translational cleavage of picornaviral polyprotein[42]	Reduces duration of symptoms if given as one lozenge every 2 hours while awake
Hot soups	Increase nasal mucus velocity	Chicken soup appears to be, particularly, efficacious
Cessation of smoking	Unknown	Reduces duration of symptoms
Local hyperthermia	Antiviral effect	See text

* As part of a proprietary formula

involving 83 volunteers, the incidence and severity of colds was reduced, as was the duration of virus shedding [66]. In those receiving interferon the mean total dose was 3.53×10^7 Units, given as a course three times daily over 4 days. This dose regimen was well tolerated. This was not, however, a truly therapeutic trial in that the virus challenge was not made until 4 hours after the fourth dose of interferon. Similar results were obtained in another placebo-controlled study of 55 volunteers with intranasal recombinant α-2b interferon [167]. In this latter study, 2×10^6 i.u./day were given for 15 days; some 19 of 26 (73%) of the placebo arm had a symptomatic cold, compared with 12 of 29 (41%) of those that received interferon. The severity of colds was also diminished and the mean duration of colds was reduced from over 4 days to 0.5 day. There was a statistically insignificant increase in bloody nasal mucus in the group receiving interferon but more unpleasant side effects were not noted.

Another immunomodulator, a thioguanosine derivative, appeared to enhance the resistance of mice to coronavirus infection but did not appear to have the same effect in man at the doses used [67].

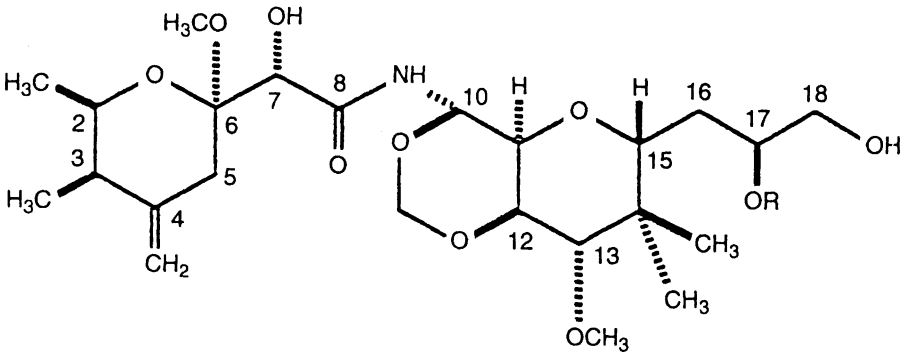
Although no trials have been attempted in humans, there are a number of substances which appear to have *in vitro* activity against coronaviruses. The protease inhibitor, leupeptin, inhibits the growth of 229E virus in MRC-c cells if given within 2 hours of infection. The IC_{50} in a plaque reduction test was shown to be 0.4 μg/ml, whereas the growth of the MRC-c cells was unaffected by 50 μg/ml [6]. Another protease inhibitor, cystatin C, has been shown to inhibit both 229E and OC43 virus [34]. Antipain had a lesser activity but other inhibitors, such as pepstatin A and cathaipsin, had no effect. Protease inhibitors have, until now, found greater use as a means of studying the infectious cycle of 229E virus than as possible antivirals.

It has been a recent vogue to investigate the possibility of natural products as antimicrobial substances, and one such compound has been discovered that has anticoronaviral activity. An extract from the *Mycale* sponge, mycalamide A (Figure 9.5), protected four of eight mice from lethal A59 virus infection, whereas all eight control mice died [136].

It is hoped that some of these substances, or derivatives of them, will be developed in the near future as antivirals. With the advent of rapid diagnostics, their use in patients with chronic lung disease is now practical.

9.10 CONCLUSIONS

The use of animal coronaviruses to study viral pathogenesis has been successful for many years. The human coronaviruses are, however, under-investigated but this may change with the recognition that they may be associated



Structure of mycalamide *A*: *R* = *H*

Figure 9.5 The structure of mycalamide.

with serious disease. The next decade should see more advances in our understanding of this group of viruses.

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