

Human Coronaviruses: A Brief Review

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INTRODUCTION

At the beginning of this century, most of the effort aimed at defining the cause of common colds was spent searching for a bacterial aetiology. It was not until the work of Dochez and his colleagues at the Rockefeller Institute in New York in the 1930s that viruses were considered to be the likely causative agents.¹ Their work with chimpanzees and human volunteers showed that filtered nasal secretions were able to cause colds in inoculated chimpanzees and isolated volunteers. The next major advance awaited the development of cell culture techniques in the 1950s when Enders showed that poliomyelitis virus could be propagated in human kidney cells. This technique was applied to the isolation of common cold viruses, and in 1956 ECHO 28 (also initially called J.H. or 2060) was isolated in monkey kidney cells;² this virus is now known to be a rhinovirus. By 1965, it was known that as well as rhinoviruses, adenoviruses, myxoviruses (influenza and parainfluenza viruses and respiratory syncytial virus) and enteroviruses were all causative agents of the common cold. Yet, a substantial proportion of colds seemed not to be due to any of these. Using human fetal tracheal organ culture, Tyrrell and Bynoe in Salisbury cultivated a virus that they termed B814 agent from the nasal washing and swab taken from a schoolboy with a cold in 1960.³ This nasal washing, number B814, was able to cause cold symptoms in 5 of 11 volunteers inoculated intranasally. B814 agent was ether labile and could pass through a bacteria-tight filter. It could reduce the ciliary activity of organ tissue, and could also be detected by virus interference in these organ cultures. At the same time as the Salisbury team were discovering B814, Hamre and Procknow in Chicago were reporting another new virus cultivatable in human embryo kidney cells from nasal secretions collected from a group of medical students in 1962.⁴ Virus was grown from five individuals, four of whom had been suffering from a cold. One isolate from student specimen 229E was chosen as the prototype strain and further characterised. It was shown to be ether-labile, about 89 µm in diameter, and had an RNA genome. Electron microscope examination of both B814 and 229E viruses showed them to be morphologically identical to avian infectious bronchitis virus and mouse hepatitis virus. These

Abbreviations used: HCV, human coronavirus; CVLP, coronavirus-like particles; HECV, human euteric coronavirus; MS, multiple sclerosis; BCV, bovine coronavirus; HEV, haemagglutinating encephalomyelitis virus; IBV, infectious bronchitis virus of avians; TGEV, transmissible gastroenteritis virus; FIPV, feline infectious peritonitis virus; MHV, mouse hepatitis virus.

viruses were subsequently termed coronaviruses because of the crown of surface projections that they possessed.⁵

THE CORONAVIRUS FAMILY

Since the late 1960s there have been three reports of the coronavirus study group, a 'vertebrate virus' subcommittee of the International Committee on Taxonomy of Viruses, and there are now a large number of viruses accepted into the monogeneric family *Coronaviridae*;⁶ there are also several viruses which are putative coronaviruses. The accepted viruses and the host species are listed in Table 1. All the viruses are host-specific and cause respiratory or gastrointestinal disease, and some are also responsible for disease of the nervous system. The basic structure and biology of all coronaviruses are essentially the same.

There are only two sero-groups of human coronavirus (HCV) that have been characterised in detail: these have HCV-229E and HCV-OC43 as the representative strains. Other strains mentioned in the literature include B814, EVS, LP and 692 and several OC (for 'organ culture') strains.

Strain B814 was the first human coronavirus isolated³ but was lost in the laboratory before it could be fully characterised.

Strain 229E was an original isolate from the study of Hamre and Procknow,⁴ and is now taken as the type strain of a group of related viruses. It has been subsequently detected worldwide in numerous sero-epidemiological studies.

Strain OC43 was isolated from a nasopharyngeal washing by McIntosh and his colleagues in 1966.⁷ It was one of six strains isolated from patients with colds that had been adapted to grow in human embryonic cell culture. Two of these, OC43 and OC38, were shown to cause an encephalitic illness, manifest as general lethargy, tremors and rigidity in suckling Swiss mice of the CD-1 strain. On the first passage the clinical illness was manifest after 11–15 days post-intracerebral inoculation but this incubation period became progressively shorter after each passage and the illness progressively more severe; after the fourth passage the mice died, 48–60 h after inoculation. Histology showed round cell infiltration of the meninges and perivascular spaces of the cortex. The liver, heart and lungs were unaffected. Since the original report this virus strain has also been found to be distributed worldwide. HCV-OC43, is, like HCV-229E, taken to be the type strain of a group of several related viruses.

Strain 692 was isolated by Kapikian's group in 1966 from a 29-year-old male with a cold and was later shown to be unrelated to HCV-229E or HCV-OC43 by immune electron microscopy.⁸ No further published data are available on this virus.

PUTATIVE STRAINS

These are listed in Table 2, and discussed below.

Tettnang virus was isolated from the cerebrospinal fluid of a 1-year-old girl with rhinitis, pharyngitis and mild encephalitis.⁹ The virus was, however, isolated only by culture in suckling mice brain tissue, and serologically seems to be a murine coronavirus.

Coronavirus-like particles (CVLPs) have been found in several disease states by electron microscopy. The most frequent association is with diarrhoeal disease. These human enteric coronaviruses (HECV), as they have been termed, have been found in many parts of the world (a review of early work was published in 1982¹⁰). There has been difficulty with defining their role in gastrointestinal disease for a variety of reasons which are discussed later. There is still doubt that they are even viruses, and it has been suggested that they may be duodenal brush border vesicles¹¹ or eukaryotic cells.¹² This ambiguity arises because they have only been characterised by electron microscopy, and the reported structures are pleomorphic. It has been suggested that some of the particles resemble toroviruses but there is no other evidence that HECV belongs to this family. The main difficulty in characterisation is that, except in a few reports, there has been a lack of success in propagating HECV. One laboratory has had some success using human fetal intestinal organ culture, which interestingly resulted in less pleomorphism of the particles.¹³ They have also been reported to grow, at least initially, in HRT-18 cell monolayers, a cell line derived from a human rectal adenocarcinoma. Continuous culture has not been possible with either cell line. The peptide profiles of HECV have been examined in polyacrylamide gels,^{14,15} with and without immunoblotting, but there is little agreement even concerning the exact number of polypeptides; the range seems to be 4 to 48, this last figure would suggest a complexity greater than poxviruses, if true.

Support of the notion that HECV is a coronavirus has come from two studies. The Italian isolates, but not the Australian, serologically cross-react¹⁶ with HCV-OC43. Second, histological examination of a fatal case of gastroenteritis showed particles present in the cytoplasm of intestinal cells, this was taken to be suggestive of replication of HECV in the cytoplasm, a feature characteristic of coronaviruses; interestingly, similar particles were not visible in the respiratory tract.¹⁷

CVLPs with other disease associations have also been reported and will be discussed later.

ANTIGENIC RELATIONSHIP OF HCV TO ANIMAL CORONAVIRUSES

The first evidence that human coronaviruses share some antigenic features with the animal viruses was reported

Table 1. Coronaviruses

Virus	Host	Relatedness to human virus
Avian infectious bronchitis	Chicken	OC43
Transmissible gastroenteritis	Pig	229E
Haemagglutinating encephalomyelitis	Pig	OC43
Mouse hepatitis	Mouse	OC43
Feline infectious peritonitis	Cat	229E
Neonatal calf diarrhoea	Cattle	OC43
Bovine enteric coronavirus	Cattle	
Rat coronavirus	Rat	
Bluecomb disease	Turkey	
Acute enteritis virus coronavirus	Dog	
Canine enteric coronavirus	Dog	
Rabbit cardiomyopathy virus	Rabbit	
Rabbit enteric coronavirus	Rabbit	
Equine enteric coronavirus	Horse	
Ovine enteric coronavirus	Sheep	
Simian enteric coronavirus	Monkey	

Table 2. Putative strains of human coronavirus

Putative strain	Disease association	Detection method
Tettnang	Respiratory infection	Culture in suckling mice brain
HECV	Diarrhoea	Electron microscopy of faeces Cell culture Serology
HECV	Necrotising enterocolitis	Electron microscopy of faeces
HECV	Tropical sprue	Electron microscopy of faeces
SD and SK	Multiple sclerosis	Culture in suckling mice brain
CVLP	Hepatitis/cirrhosis	Electron microscopy of serum Serology
CVLP	Nephropathy	Electron microscopy of kidney tissue Serology

even before the former were described. In 1964 antibodies to various murine coronaviruses (MHV-A59, MHV-5, MHV-1, MHV-3 but not MHV-JHM) were shown to be present in the sera of US marine recruits and the children of naval personnel by a complement fixation test and/or a plaque neutralisation test.¹⁸ On the basis of later work by others it is likely that these were anti-OC43 antibodies.¹⁹

Sera reacting with HCV-OC43 also seem to have reactivity^{20,21} with bovine coronavirus (BCV), haemagglutinating encephalomyelitis virus (HEV) and infectious bronchitis virus (IBV). HCV-229E has been shown to have bilateral cross-reactivity with transmissible gastroenteritis virus (TGEV), feline infectious peritonitis virus (FIPV) and mouse hepatitis virus type 3 (MHV-3). The last is, however, thought to be brought about by the adherence of bovine serum components, since growth of the virus in serum-free culture abolishes the cross reaction. HCV-229E has also been reported to cause a cardiomyopathy in rabbits,²² but it is probable that this is due to a related rabbit coronavirus. There is no demonstrable antigenic relationship between HCV-229E and HCV-OC43.

Recent sequence data of the genes coding for N²³, M²⁴ and HE²⁵ proteins of HCV-OC43 have confirmed the serological relationship between this virus and BCV and MHV. There also appears to be a common ancestry, phylogenetically, between HCV-OC43 and influenza C virus.²⁵ Sequence data of the N^{26,27} and S²⁸ proteins of HCV-229E show limited homology with TGEV and FIPV, but greater predicted amino acid sequence matching in specific regions.

Most other human coronaviruses described are antigenically related to either HCV-229E or HCV-OC43, with the exception of strain 692 which has been shown to be unrelated to either. Strain B814 was not antigenically characterised.

STRUCTURE AND PROPERTIES OF HUMAN CORONAVIRUSES

Structure

Coronaviruses are large enveloped viruses of 80 to 200 nm in diameter as measured by electron microscopy of negatively stained virions; the measured diameter of HCV-229E virions is significantly greater if the staining is by uranyl acetate than by potassium phosphotungstate.²⁹ They have a single-stranded RNA genome of positive polarity, and a buoyant density in sucrose of 1.18 g/ml. The characteristic morphological feature of all coronaviruses is the presence of a fringe of about 200 club-shaped projections of 10–20 nm in length that form the 'corona' that gives the viruses their name. A general representation of a coronavirus is shown in Figure 1. The various structural components will now be discussed in detail; it should be noted that, despite the wealth of information on the physical components of human coronaviruses, the biological properties of these components have not been extensively investigated and are assumed to be similar to that shown for other coronaviruses; there is much evidence, however, to suggest that this assumption is reasonable. For a detailed review (and references) of the organisation and replication of coronaviruses the reader is referred elsewhere.³⁰

Human coronaviruses have been noted to have only one type of surface projection, these peplomers are 20 nm long and about 7 nm wide at the tip. They are formed by a glycoprotein (termed S) of M_r 160 000–200 000. It is thought to be a dimer of two dissimilar proteins, which form tetramers to produce the surface peplomer or spike.

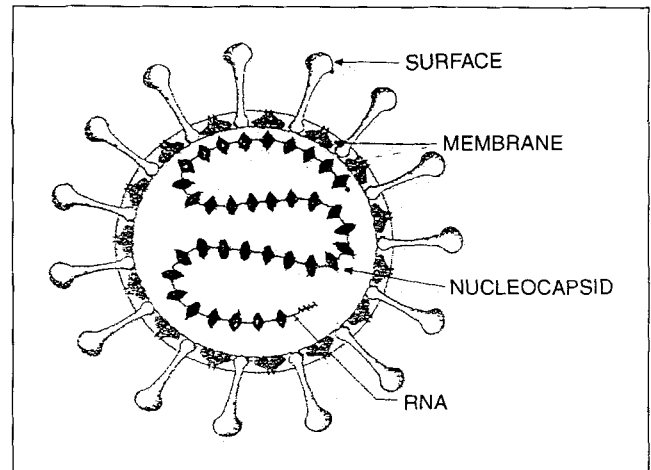


Figure 1. Basic structure of coronaviruses.

The spike can be removed from the rest of the virion by digestion with bromelain, and can then be separated into the distinct proteins of the dimers by digestion with trypsin. The predicted amino acid sequence of the surface protein from several strains of coronavirus is now available and the general structure seems to be of an N-terminal signal sequence, a membrane-spanning domain, and a C-terminal hydrophilic sequence; there are also a large number of glycosylation sites which in MHV have glucosamine and fucose residues. Mainly on the basis of experiments using specific monoclonal antibodies it is thought that the surface protein is responsible for the binding of the virus to cell surface receptors (and haemadsorption), inducing neutralising antibody, eliciting cell-mediated cytotoxicity, inducing cell fusion and the pH-dependent thermolability of coronaviruses.

The most abundant protein is the membrane protein (designated M), which is present in approximately twice the amount of the nucleocapsid protein, 16 times the amount of the peplomeric protein, and over 30 times the amount of the HE protein. The M protein is really a set of four proteins (or complexes) of approximate M_r 20 000, 24 000, 27 000 and 40 000 in human coronaviruses. The resultant protein size depends on the degree of glycosylation and on whether a dimer is formed. It is mainly embedded in the viral envelope but about 10% of the N-terminus end protrudes from the surface, and the C-terminus protrudes interiorly. There are differences in the composition of this protein in the two main groups of human coronaviruses, as exemplified³¹ by the aggregation of HCV-OC43 M protein on heating in sodium dodecyl sulphate, but not that of HCV-229E. The M protein is essential for viral budding, forms the viral envelope and interacts with the nucleocapsid, possibly anchoring the genome to the viral envelope.

The nucleocapsid protein (designated N) of M_r 47 000–55 000 is nonglycosylated and basic, though it has an acidic C-terminus. Analysis of all the nonhuman mammalian coronaviruses so far has shown that it is also serine-rich. Its main role is to encapsidate the RNA genome, but evidence from studies with MHV, in which N-specific antiserum was added to an *in vitro* replication system, has shown that it may be important in RNA replication; in particular, the synthesis of genome-length RNA.

In addition to the three main structural proteins possessed by all coronaviruses, human coronaviruses (as well as HEV, MHV, MHV-JHM strain and BCoV) possess a fourth protein, a haemagglutinin/esterase, termed HE, which also resides in the envelope. It is glycosylated, has a M_r of 63 000, exists as a dimer linked by a disulphide bridge and seems to be responsible for haemagglutination in the case of HCV-OC43. It may also play a role in binding to cell surfaces, and in BCoV has been shown to have acetyl-esterase receptor destroying activity. Several minor proteins have been described in human coronaviruses, but no role has been ascribed to them, and indeed, it is now thought that some, if not all, of these polypeptides found in gel analyses are subcomponents of the four major proteins.

The genome of coronaviruses is a single strand of positive-sense RNA that in human coronaviruses appears to have a M_r of about 6 million when run with known molecular weight markers in gels; this may be an underestimate as it was for IBV which has now been sequenced and found to be 27 kilobases (kb) long, the largest viral RNA virus genome determined so far. It has a polyadenylated tail at the 3' end, and is infectious. It is heat labile, tending to fragment above 60°C. It is intimately associated with the nucleoprotein and can be seen under the electron microscope to be in a helical arrangement.

Nucleotide sequence data are now available from over 6.7 kb of the 3' end of the virus of HCV-229E which covers the open reading frames for mRNAs 2 (translated to produce S protein),²⁸ 3, 4, 6(M protein)^{32,33} and 7 (N protein);^{26,27} mRNA 4 has two open reading frames which potentially code for a 44 amino acid polypeptide of M_r 4600 and a polypeptide of 83 amino acids (M_r 9000) which is basic and leucine-rich.^{34,35} The nucleotide sequences of the genes coding for the membrane, surface and haemagglutinin/esterase proteins of HCV-OC43 are also now available and predict structures similar to those of related animal viruses.

The envelope of coronaviruses is thought to be derived from the internal membranes from which they bud (see replication strategy), and indeed experiments with TGEV have shown that the lipid composition of the virus is dependent on the cells it is grown in, and accurately reflects the lipid composition of these cells. There may be a biological advantage for viruses budding from internal membranes over those budding from plasma membranes, as the former membranes are less susceptible to solubilisation by bile salts and other detergents, thus conferring a survival advantage in the intestinal tracts of hosts. Other host-derived components have been described for various coronaviruses, including cellular proteoglycans, but it is difficult to ascertain whether these are artefacts of the virus purification procedures.

Properties

Despite very similar structures, the two main types of human coronaviruses have different physical properties. Both viruses are inactivated at both 33°C and 37°C, in the presence of the appropriate maintenance medium and 2% fetal calf serum, but in the absence of host cells. HCV-229E is more labile than HCV-OC43, in that at 33°C there is less

than 1% of the original infectious virus after 10 h, whereas it takes 40 h to achieve³⁶ the same reduction with HCV-OC43. HCV-229E appears, however, to retain infectivity³⁷ despite repeated cycles of freeze-thawing if maintained at 4°C. HCV-OC43 is also more stable to lowering the pH of the medium than HCV-229E. Similar reduction curves to that of temperature were found in the same study when ultraviolet light was used to inactivate the viruses; an unusual finding was that they were, relative to other viruses, resistant to ultraviolet light because the viruses tended to clump in the absence of bovine plasma albumin. HCV-229E is also susceptible to inactivation by 3% hydrogen peroxide and 0.2% C4 (a quaternary ammonium compound that has been used as a viral disinfectant) within 1 minute of application;³⁸ like HCV-OC43, the strain is also sensitive to lipid solvents and detergents, such as chloroform.³⁹ An unexpected finding is that the growth of HCV-229E in L132 cells can be inhibited⁴⁰ by actinomycin D, especially if applied to the culture early in the infection cycle; it is presumed that this is because of inhibition of some host-virus interaction, since other coronavirus-host systems are not similarly affected.

It has also been shown that the growth in WI-38 cells was best, for both viruses, between 32°C and 34°C, and raising the temperature to 37°C resulted in a complete absence of detectable virus growth.³⁶ The peak viral titres of HCV-229E in human embryonic lung diploid fibroblast cells at 35°C are obtained at 22–48 h, then there is a rapid decline in infectious titre; only after this period is any cytopathic effect discernible. This makes the virus difficult to passage to high titres; similar results have been shown in L132 and HeLa cells.

Another investigated property of HCV-OC43, is the ability of this virus to haemagglutinate human group O, chicken, mouse, rat and vervet monkey erythrocytes.⁴¹ This haemagglutination can be reversed by heating, as well as pre-treatment of the virus with trypsin, ether and Tween-80. The property of temperature-dependent haemagglutination has been exploited in purification of the virus. HCV-OC43 also nonspecifically haemadsorbs to rat and mouse erythrocytes if the virus has been grown in BSC-1, HEV, MK or WI-38 cell culture. This nonspecific haemadsorption is due to the presence of a high-density lipoprotein inhibitor in fetal calf serum. HCV-229E has not been shown to have haemagglutinating properties, despite the presence of the HE protein. It does, however, adhere to sheep red cells treated with tannic acid and this property has been used to develop an indirect haemagglutination test.⁴²

REPLICATION STRATEGY AND MORPHOGENESIS OF CORONAVIRUSES

Most work on the molecular biology of coronaviruses has been done using MHV or IBV as the models but it would be expected that an essentially identical replication strategy is employed by the human viruses. A schematic diagram of the basic replication strategy is shown in Figure 2.

HCV-229E has been shown to attach to the surface of MRC-C cells randomly when these cells have been fixed or

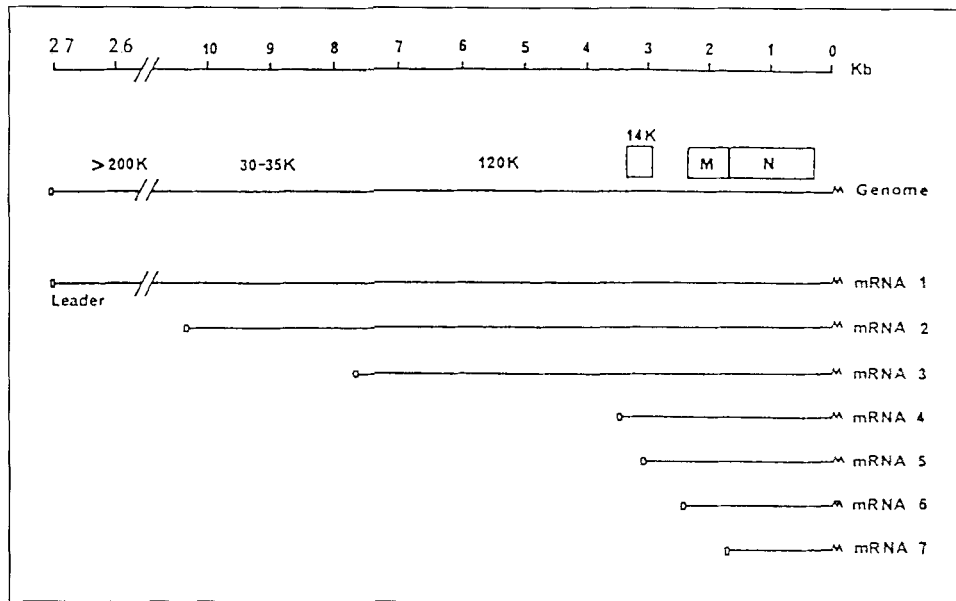


Figure 2. RNA species of coronaviruses. The coding regions for M and N are shown (see text for details).

exposed to virus at 4°C. These particles, however, redistributed on warming.⁴³ Though this might suggest that specific receptors do not exist, other evidence has suggested the contrary viewpoint. First, coronaviruses show marked tropism for certain cell types. Also attachment of MHV to L2 cells can be abolished by prior treatment of the cells with protease, suggesting an essential protein for binding. Furthermore, results from a Japanese study⁴⁴ have shown that there is a genetic factor that is important in determining the susceptibility of cells to HCV-229E. In this study HCV-229E was shown to replicate in hybrids of three mouse cell lines (RAG, LM/TK-, A9) and human WI-38 cells. The virus would not, however, replicate in hybrids that lacked the q11-qter region of human chromosome 15, suggesting the production of some protein that is important for susceptibility to HCV-229E, conceivably a receptor. This receptor protein has recently been identified⁴⁵ as human aminopeptidase N. This protein is a cell-surface metalloprotease that can be found on lung, intestinal and kidney epithelial cells. This further strengthens the relationship between TGEV and HCV-229E as the former uses the porcine aminopeptidase N as a receptor. HCV-OC43 does not bind to this protein but recognises receptors containing sialic acid similar to those recognised by influenza C.⁴⁶

Once attached, the virus enters the cell and then uncoats; there are little data on this stage of virus replication. Electron microscope studies have suggested that some coronaviruses may enter the cell via clathrin-coated pits. After uncoating, the rest of the viral replication cycle takes place entirely in the cytoplasm. The RNA genome of coronaviruses contains 5 to 7 open reading frames for the production of a series of subgenomic mRNAs. The positive-stranded genome, initially, serves as a template for the production of a full length negative-stranded intermediate by an RNA-dependent RNA polymerase. This polymerase is thought to be coded for by the virus and may be distinct from the polymerase that is responsible for the production of the subgenomic mRNAs that are transcribed from the negative-

stranded intermediate. These subgenomic mRNAs are coterminal at the 3' end, have a polyadenylated tail and a common 5' leader sequence. The presence of the leader sequence is explained by the hypothesis that transcription is discontinuous, the RNA polymerase reading the first 60–70 bases of the 3' end of the negative-stranded template then transcribing the appropriate mRNA sequence. The unique region at the 3' end of each mRNA codes for the protein, which is then post-translationally glycosylated. In all the coronaviruses, so far studied, the smallest mRNA codes for the nucleocapsid protein. RNA and protein synthesis take place entirely on internal membranes, the Golgi apparatus and rough endoplasmic reticulum.

Scanning electron microscopy reveals no changes in the morphology of HCV-229E infected cells for 6 h. Then virus particles can be seen within cytoplasmic vacuoles, which increase in number for the next 6 h. Twelve hours after infection, virus particles can be seen on the outside of cells. The process of transport to the plasma membrane and the budding process still require investigation. HCV-OC43 has been shown to have a similar process of maturation, but over a slightly longer time scale.

HOST RESPONSE

Currently, there is no evidence that human respiratory coronaviruses spread from the respiratory mucosa. The data suggest that there is an effective local immune response.

It has already been mentioned that there may be a genetic component determining the susceptibility to coronavirus infection. Further evidence for the possible importance of genetic susceptibility comes from a study of 84 monozygotic twins, in whom susceptibility to the common cold with respect to the alpha-1-antitrypsin phenotype was examined.⁴⁷ Those twins who had phenotypes, MS and MZ, and thus had lower enzyme levels compared with the normal MM phenotype, were 1.2 and 2.5 times, respectively, more likely to suffer from the common cold. It is not

suggested that this particular gene is involved directly with susceptibility, but it may be a factor, or linkage marker of the relevant genes. It should also be noted that the particular virus group to which susceptibility was increased was not determined.

Humans, in common with many other mammals, have been shown to have a nonspecific serum inhibitor of HCV-OC43 and BCV infectivity.⁴⁸ This inhibitor was shown to be effective at neutralising HCV-OC43 infectivity in the murine model, and it is conceivable that it is a factor in preventing systemic spread of human coronaviruses. The specific humoral immune response is mainly directed at the surface projection; this has been shown by looking at convalescent sera in volunteers taken 21 days after challenge with the virus, though antibodies directed against the two other major proteins can be found in both humans and rabbits.⁴⁹ Experiments in volunteers have also shown that both specific IgA in nasal secretions (and also presumably cell-associated IgA), and a high concentration of specific IgG in the serum were both important for the prevention of infection; and for the attenuation of symptoms once the volunteer was infected. It appears that the level of mucosal specific IgA is more important than the level of circulating specific IgG in determining the duration of virus shedding.⁵⁰ It should be noted, however, that re-infection is common despite the presence of pre-existing antibody. The role of IgE⁵¹ and antibody-dependent cytotoxicity⁵² has also been examined, but the significance of these in the immune response to infection is unclear.

CLINICAL FEATURES

Mode of transmission

It is generally assumed that common cold viruses are transmitted by aerosols but this is disputable. An editorial⁵³ in the *Lancet* in 1988 summarises the research into the two alternative hypotheses, namely, aerosol spread and direct transmission by fingers or fomites. It is possible to spread colds from infected volunteers to uninfected volunteers, though often with some difficulty, with the two groups separated either temporally or spatially; HCV-229E has also been shown to survive airborne for days at medium to high humidity.⁵⁴ It has, however, also been shown that rhinoviruses could survive on hands and inanimate objects and thus allow transmission in that manner. The likelihood is that both routes may occur but, *in vivo*, transmission by aerosol is the common one.

Common colds

Coronaviruses are thought to cause up to 30% of all colds. The peak incidence seems to be during the period from the end of autumn to the beginning of spring when rhinovirus colds, which predominate at other times of the year, are relatively infrequent. Prevalence of antibodies to one or other human coronavirus is very high, being 6%–37% in infants less than 1 year of age, 54%–80% in the 1–5 year age group, and 100% in those over 5 years of age; this figure drops slightly with adult populations.^{55–59} Outbreaks of coronavirus infection seem to show 2–3 year

cycles, HCV-229E epidemics alternating with HCV-OC43 infection.

After an incubation period of 2–4 days, an illness comprising general malaise, headache, profuse rhinorrhoea, nasal blockage and sneezing is produced. HCV-OC43 infection also tends to result in a cough and pharyngeal hyperaemia, this being a less common feature of HCV-229E infection. Both can produce a fever, enlargement of the cervical glands, and gastrointestinal symptoms (abdominal pain or diarrhoea). This illness lasts from 3 to 18 days, with a mean duration of 7 days. In serological surveys and volunteer studies, 30% of infected individuals remain asymptomatic.

Lower respiratory tract infection

Coronaviruses have also been associated with lower respiratory tract illness in sero-epidemiological studies,^{60–63} though it should be noted that in one early study there was a higher incidence of coronavirus infections in the control group than in the diseased. The peak incidence of HCV-229E lower respiratory tract infection seems to be in preschool children, and is rare in infants and adults; it presents as croup, bronchitis or pneumonia. In a 3-year study in the USA coronaviruses were found to be the third most common cause of bronchiolitis and pneumonia, after para-influenza 3 and respiratory syncytial virus, in 417 children under the age of 18 months. HCV-OC43 is more likely than HCV-229E to cause lower respiratory tract infection in adults. Asthmatic children and the elderly seem to be at particular risk of lower respiratory tract infection, and recent data would suggest that coronaviruses are the second or third most frequently identified agent responsible for precipitating acute wheezing in asthmatic children (unpublished data).

Other clinical illnesses

Associations of human respiratory coronaviruses with other clinical syndromes have been reported in the literature, usually on the basis of a serological response in the patient. The evidence is, at best, tenuous in most cases. A serological survey has also shown an increased prevalence of anti-HCV-229E antibodies in patients with nasopharyngeal carcinoma.⁶⁴ It should be noted that these patients also had antibodies reacting with Epstein–Barr virus nuclear antigen, and the authors merely suggest that RNA viruses may play an uncertain role in pathogenesis. There are also case reports of patients with carditis and thyroiditis who had a rise in antibody titres to HCV-OC43, both from the same laboratory in Finland.⁶⁵ An increased level of CSF antibody to OC43 has also been reported in patients with Parkinson's disease than in controls.⁶⁶ No other evidence has been reported to substantiate anything other than an association in these cases.

Disease associations of coronavirus-like particles

If CVLPs are accepted as an entity, there are still conflicting data linking them to disease. As already stated an association with gastrointestinal illness has been claimed but there must be doubt about this being aetiological. There

are wide differences in the published prevalence rates of CVLPs in both diarrhoeal, and nondiarrhoeal populations. A study of 137 homosexual men with symptomatic HIV infection (a group which might be expected to have a high carriage rate) revealed only two cases of CVLPs, and only one of these was symptomatic.⁶⁷ This contrasts with 69.8% in 862 diarrhoeal specimens collected over an 8-year period in another USA study;¹⁵ this last figure might be taken as supportive of the role of these particles in causing diarrhoea, but other, case controlled, studies have not shown such high prevalence rates. Specific groups that have been shown to have high carriage rates are Australian aborigines,⁶⁸ particularly infants outside the neonatal period, and homosexual males.^{69,70} In two studies, 35% and 50% of male homosexuals were found to be excreting the particles. Animal studies have also shown high carriage rates in calves and nonhuman primates; this could be a factor explaining the high prevalence in some areas. It is apparent that much more work needs to be done before CVLPs, and their role in causing diarrhoea, is defined.

Two other gastrointestinal disease associations have been reported. In a Parisian study of 91 neonates, 47 of which were controls without diarrhoea, 32 newborns had necrotising enterocolitis and 23 of these had detectable HECV by electron microscopy in their faeces.⁷¹ None of the controls had detectable HECV and the only other viruses found using electron microscopy in the NEC cases were rotaviruses, but only in association with HECV. HECV particles were also detected in a large proportion of contacts. This study has not been repeated. Similarly, there is an isolated report of HECV detected in the stools of a 47-year-old Indian male with tropical sprue.⁷² It was also detected in intestinal biopsy material.

The second most investigated disease association of CVLPs is with neurological disease. In 1976, a report of intracisternal particles seen in post-mortem tissue sections of the brain of a multiple sclerosis (MS) patient first linked human coronaviruses to demyelinating disease.⁷³ Prior to that it had already been established that murine coronaviruses could induce demyelination in mice. These particles were 55–65 nm in diameter and were found only in the cisternae of the rough endoplasmic reticulum. It was because of their morphology and this cytoplasmic location that these particles were tentatively described as coronaviruses. No further evidence was forthcoming until 4 years later, when two coronaviruses, termed SD and SK after the initials of the patients, were isolated from the brains of two patients with multiple sclerosis.⁷⁴ These were characterised as coronaviruses by electron microscopy. Doubt was cast as to their existence as human viruses, however, as viruses were only recovered after passage in, first, suckling mice brains and then a murine cell line; neither isolate would grow in human cell lines. In defence of the notion that they are human viruses was serological evidence that showed no reactivity of either the mice or the cell lines with polyclonal anti-MHV sera. Moreover, neutralising antibody was found in both CSF and serum of the patients to one or both isolates. It could also be shown by plaque neutralisation and immunoprecipitation studies that the isolates were related antigenically to HCV-OC43 but not HCV-

229E; they were also related to the murine strain A59. Later studies have confirmed the serological relationship of SD and SK to HCV-OC43 but also to other murine coronaviruses.⁷⁵ The nucleotide sequences of both SD and SK have now been compared with MHV-A59 and they share extensive homology with this strain but not with other murine, or human, coronaviruses. Serological surveys of multiple sclerosis patients have not, with one or two exceptions, supported the relationship between coronaviruses and multiple sclerosis but there are other data that suggest the hypothesis should not be dismissed. Intrathecal antibody, reactive with both HCV-OC43 and HCV-229E, has been found in cerebrospinal fluid from multiple sclerosis patients more frequently than in sex-matched and age-matched controls.⁷⁶ This is not necessarily indicative of coronaviruses being aetiologically related to the disease, however, since intrathecal synthesis of antibody to a number of viruses has been shown in multiple sclerosis patients and it may just be a feature of the autoimmune nature of the disorder.

More specifically, nucleic acid probes have been used recently to detect coronavirus genome in CNS tissue.⁷⁷ Using labelled cDNA of the putative SD strain as a probe, and *in situ* hybridisation techniques, SD genome was detected in 9 of 21 MS post-mortem CNS specimens; a positive signal was obtained in only 1 of 16 specimens from control patients without neurological disease, and one specimen from control patients with other neurological disorders. HCV-OC43 has not been detected using similar methods. Corroboratory evidence has come from cell culture studies in which it has been shown that HCV-OC43 can cause a persistent infection in a human glioblastoma cell line, and thus exhibits neurotropism;⁷⁸ it has also been shown that human fetal brain cells are susceptible to HCV-OC43 but without the production of infectious virus. A recent report has offered a different approach to investigating the role of coronaviruses in multiple sclerosis. HCV-OC43 was not detectable by reverse transcriptase-polymerase chain reaction (RT-PCR) in the brains of multiple sclerosis patients but HCV-229E RNA was found in 4 of 11 specimens,⁷⁹ HCV-229E RNA was not detectable in controls. In support of the relationship of HCV-229E with multiple sclerosis is the finding that a sequence of five amino acids, predicted from the nucleotide sequence of gene 4, is homologous with an immunologically important region of human myelin basic protein.³⁵

CVLPs have been found in the sera of patients with chronic active hepatitis who had no other known aetiological agent detectable,⁸⁰ such as the hepatitis B virus. These particles have also been seen in healthy individuals and it is unlikely that they play an aetiological role in the disease.

Coronavirus-like particles have also been associated with renal disease. They have been seen, under the electron microscope, in renal biopsy patients from patients suffering from endemic nephropathy, also called Balkan nephropathy because it was only found in Yugoslavia, Bulgaria and Rumania.⁸¹ This is a disease that progresses slowly to uraemia and because it tended to cluster in both families and communities, an infectious aetiology was thought likely. The particles seen within cytoplasmic vesicles have

not, however, been successfully propagated and sero-epidemiological surveys looking for an immune response to porcine, avian and human coronaviruses have not shown an increased prevalence of antibodies to coronaviruses in patients suffering from Balkan nephropathy. It should be noted that apart from the human enteric coronaviruses there is little characterisation, apart from morphology, of the CVLPs associated with the nonrespiratory diseases as human viruses, so it would be premature to accept them as human coronaviruses at this time.

DIAGNOSIS

It is beyond the scope of a brief overview to provide detail about methods and references: this information is available elsewhere.^{82,83}

Cell and organ culture

The most important property for characterising these viruses has been the ability of the viruses to infect certain cell lines and organ cultures; the difficulty associated with viral culture has also been the most important reason that these viruses have not been studied easily. Initial isolation of HCV-OC43 is dependent on growth either in human embryonic tracheal organ culture or, less well, in suckling mice brain. The original isolation of HCV-OC43 was in tracheas from 5–9 months old fetuses that had been stored in cold Hanks solution with 10% fetal calf serum for no more than 48 h. The tracheas were then cut into 2–3 mm squares in Petri dishes which were then inoculated with Leibowitz medium and 0.2% bovine serum albumin. Culture of 0.2 mL aliquots of nasal washing was at 33°C on a rocker platform. The presence of replicating virus was determined by looking for cessation of ciliary movement, and comparing this with uninfected controls. Once isolated, HCV-OC43 can be adapted to grow in human embryonic lung diploid fibroblast cells, human fetal tonsil diploid fibroblasts, human embryonic heteroploid rhabdomyosarcoma, primary African green rhesus monkey kidney cells, or green monkey kidney epithelioid cells; this adaptation to cell culture is not consistently successful, and many workers have chosen to adapt isolates to suckling mice brain.

HCV-229E is more easily grown in cell monolayers. It was first isolated in human embryonic kidney cells but is now more commonly grown in human embryonic lung diploid fibroblast cells. The virus has also been successfully propagated in continuous human embryonic lung epithelial cells, human fetal tonsil diploid fibroblast cells, human embryonic heteroploid rhabdomyosarcoma cells and human embryonic nasal and tracheal organ culture. They have also been adapted to grow in human macrophages and human type II pneumocytes.

Cytopathic effects seen either in organ culture or in cell monolayers are usually not specific for coronaviruses and, except in controlled volunteer trials, an additional diagnostic step is required for certain identification of the isolate as a coronavirus: techniques used include electron microscopy, immune-electron microscopy, complement fixation, indirect fluorescence, plaque reduction neutralisation and fluorescent focus assays.

Serology

Serological tests have been employed extensively in epidemiological surveys. Serum collected is usually heat inactivated and then diluted 1:4 for use in one of the tests described below.

Single estimations are only of use in determining previous exposure, since prevalence is very high in all the populations so far studied; rises in antibody levels in paired acute and convalescent sera can be used for serodiagnosis of recent infection. Neutralisation tests have been used in those cell lines in which a cytopathic effect is readily discernible. Neutralisation tests have been described using both tube cultures and 96-well flat-bottomed microtitre well plates. Serum neutralisation tests have also been described for HCV-OC43, preventing the infectivity in suckling mice brain and BSC-1 cells. Plaque reduction serum neutralisation tests have been described for HCV-229E using L132 cells and HCV-OC43 using MA-321 cells.

Complement fixation tests for both viruses have also been used, but have an inbuilt drawback, in that complement fixing antibodies are short-lived. Crude antigen extracts of infected cells have usually been used and may have led to lack of specificity.

Haemagglutination tests have been developed for HCV-OC43, but HCV-229E does not share this property. Haemagglutination-inhibition can be examined using human vervet monkey, chicken, rat or mouse erythrocytes, but it is important that the sera are treated with phospholipase C to prevent false-positive reactions; this is probably due to a high-density lipoprotein that also acts as an inhibitor. An indirect haemagglutination test for the detection of HCV-229E antibodies is dependent on the ability of the virus to sensitise glutaraldehyde-fixed, tannic acid-treated sheep red cells.

A single radial haemolysis test using sheep erythrocytes has been used as a semi-quantitative method of antibody detection. This was found to be applicable to the detection of both HCV-229E and HCV-OC43 antibodies.

An indirect fluorescent antibody was described for both viruses by Monto and Rhodes, and an immune-adherence haemagglutination test for HCV-229E antibodies by Gerna *et al.* Neither of these tests has been further evaluated.

Another test system that has only been reported once is that of solid-phase radioimmunoassay. This was found to be more sensitive than single radial haemolysis, haemagglutination inhibition or complement fixation.

The most sensitive method described is, however, the enzyme immunoassay (EIA). This can be applied to both HCV-229E and HCV-OC43. Antigen is prepared from clarified cell-culture-derived virus (for 229E) or mouse-brain cultured virus (for OC43). Using a standard assay system, a ratio of 1.5 or greater in the OD₄₅₀ reading of paired sera is taken as evidence of recent infection. A low false-positive and false-negative rate is well documented.

Direct antigen/virus detection

The standard technique is electron microscopy. As already mentioned, the size of the virion depends on the stain employed, but the characteristic morphology is still usually

discernible. It is usually possible to detect viral particles using this technique if they are of high titre, at least 10^5 virus particles per millilitre, but in natural respiratory infections this level of virus production is not usually obtained. Immune electron microscopy enhances the method by causing virus particles to aggregate, but has only been shown to be of benefit when diagnosing HECV infections. Immunofluorescence has been used on nasopharyngeal aspirates but the low percentage of positive results suggested that there were many false-negatives. An EIA has been shown to be of greater promise.

More recently methods based on nucleic acid hybridisation have been described. The first of these was based on RNA:RNA hybridisation using radioisotopically labelled cloned probes. This method was, at least, as sensitive as cell culture and enabled diagnosis within 24–48 h. This technique has, however, been superseded by the development of gene amplification-based methods. 'Nested' RT-PCR methods using primers specific for the nucleocapsid genes of HCV-229E and OC43 are 100-fold more sensitive than the RNA probe method when applied to infected nasal aspirates; further internal probing is also unnecessary.⁸⁴

MANAGEMENT

The management of HCV-229E infection is essentially, at present, management of the common cold. This, at least to the individual, is a trivial illness and merits only symptomatic relief. Many folk remedies have been described, and there is a plethora of non-prescribed medicines available; it is beyond the remit of this review to go into these but the data concerning specific treatment will be briefly mentioned.

There has been some evidence that zinc lozenges taken every 2 h reduce the severity and duration of colds. This study has not been consistently supported, and in any case is unlikely to be of major benefit in coronavirus colds, if it is true that the mechanism of action of zinc is inhibition of post-translational viral polypeptide cleavage.

Interferon alpha has been shown in a double blind, placebo-controlled trial to reduce the severity of the clinical illness and reduce viral shedding times in volunteers with HCV-229E colds. The presence of side-effects in treating such a mild illness is, however, unacceptable.⁸⁵

The application of moist hot air to the nares to raise the mucosal temperature to 43°C initially showed promise as an effective means of reducing the severity of natural colds. This initial promise has not been substantiated but further work needs to be done.⁸⁶

Vaccines are under development but not yet available. It is likely that these will be targeted at particular groups at risk of serious illness following coronavirus infection: for example, children with asthma.

CONCLUSIONS

Human coronaviruses have until recently been greatly understudied. Recent work has concentrated on the nucleotide sequencing of the two prototype strains. This offers much useful information but if we are to understand the

biology of these viruses, and mechanisms of pathogenesis, we need good *in vitro* models. One means of acquiring these is by the development of transgenic animals, though this will still have the drawback of not, necessarily, reflecting the human situation. The best model system must still be the human volunteer and it is hoped that such research will continue in the future.

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