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Review

SARS: Lessons Learned from Other Coronaviruses

SONIA NAVAS-MARTIN and SUSAN R. WEISS

ABSTRACT

The identification of a new coronavirus as the etiological agent of severe acute respiratory syndrome (SARS) has evoked much new interest in the molecular biology and pathogenesis of coronaviruses. This review summarizes present knowledge on coronavirus molecular biology and pathogenesis with particular emphasis on mouse hepatitis virus (MHV). MHV, a member of coronavirus group 2, is a natural pathogen of the mouse; MHV infection of the mouse is considered one of the best models for the study of demyelinating disease, such as multiple sclerosis, in humans. As a result of the SARS epidemic, coronaviruses can now be considered as emerging pathogens. Future research on SARS needs to be based on all the knowledge that coronavirologists have generated over more than 30 years of research.

INTRODUCTION

SEVERE ACUTE RESPIRATORY SYNDROME (SARS) appears to have originated in Guangdong Province, China, in late 2002. It spread to locations in Asia including Hong Kong, Vietnam, Singapore, and Taiwan and in North America, most notably to Toronto. By the end of the epidemic, the CDC and WHO were reporting more than 8000 cases, with more than 800 deaths and some evidence of recurring disease (50). At present, there is no effective treatment for SARS. The epidemic has been controlled essentially by isolation. Given the fast spread of the disease, the high mortality rates (>10%, up to 40% for certain age groups), the lack of any treatment and the uncertainties of whether the disease will return and at what frequency, development of a SARS vaccine as well as anti-viral strategies are of a high priority.

A newly identified coronavirus was isolated from SARS patients (50); proof that this virus is the etiologic agent for SARS was provided by results of infections carried out in non-human primates, in which Koch's postulates were fulfilled (33). Several groups of scientists, including those at CDC, have quickly sequenced the genome of this new coronavirus referred to as SARS-CoV (81,87); the sequence information demonstrated that this is a previously unrecognized coronavirus. The identification of a new coronavirus as the etiological agent of SARS has evoked much new interest in the molecular biology and pathogenesis of coronaviruses.

Coronaviruses form a group of pathogenic, enveloped RNA viruses that have the largest genome (around 30 kb) among RNA viruses. In 1931, Schalk and Hawn published a description of "a new respiratory tract

Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

disease affecting baby chicks" (82). That discovery lead to a series of reports on animal diseases, such as the "hepatitis virus of mice" reported by Gledhill et al. in 1951 (37). The name coronavirus was introduced in 1968 by Tyrrel et al. (96); it was based on the "corona"-like morphology of these viruses observed by electron microscopy. However, it was not until 1975 that the Coronaviridae family was established by the International Committee on the Taxonomy of Viruses. Coronaviruses are divided into three antigenic groups (termed groups 1, 2, and 3), that infect many species of animals including humans (63) (Table 1). Coronaviruses infect mainly mucosal surfaces, causing respiratory and enteric diseases that may account for important economic loss, particularly in the case of porcine and bovine coronavirus infections. Of note, some coronaviruses induce hepatic and central nervous system diseases (mouse hepatitis virus, MHV) and even systemic infections (feline infectious peritonitis virus, FIPV). Under experimental conditions, susceptibility to coronavirus infections depends on factors such as the genetic background, age of the host, virus strain, dose, and route of inoculation (55,86). Until recently, there were two prototype human coronaviruses, HCoV-229E and HCoV-OC43, which, together cause about the 30% of the common colds (6,62). The newly isolated SARS-CoV is related in sequence to other known coronaviruses. SARS-CoV has been suggested to define a new fourth antigenic group of viruses (81); however, one report suggests it may be a related member ("early split-off") of group 2 coronaviruses (87) along with the prototype coronavirus, murine hepatitis virus (MHV) and the human cold virus OC43. SARS-CoV is most closely related in sequence to group 2 coronaviruses, including murine coronavirus, mouse hepatitis virus (MHV), human coronavirus OC43 and bovine coronavirus (BCV) (47,81,87,98,99). This would suggest that the information gathered with MHV and other coronaviruses may be quite important and relevant to the understanding of the SARS-CoV.

CORONAVIRUSES

Taxonomy. Coronavirus taxonomy has recently been updated (27,29). Coronaviruses together with toroviruses (that cause enteric diseases in cattle and possibly in humans) form the *Coronaviridae* family,

TABLE 1. CORONAVIRUSES HOST TROPISM AND DISEASES

			Disease		
Host	*Group*	*Virus*	*Respiratory*	*Enteric*	*Other*
Human	2	HCoV-OC43,	+	(+)	
	1	HCoV-229E,	+	—	
	?	SARS-CoV	+	(+)	Kidney
Chicken	3	IBV	+	+	Kidney
Turkey	2	TCV	+	+	
Cat	1	FIPV	+	+	Systemic
Cat	1	FECV	—	+	
Dog	1	CCV	+	+	
Pig	1	TGEV	+	+	
Pig	2	HEV	+	+	CNS
Cow	2	BCV	+	—	
Rabbit	2	RbEVC	—	+	
Rat	2	SDAV	—	—	CNS
Mouse	2	MHV	+	+	CNS

HCoV-OC43 and HCoV-OC229 are the previously known human coronaviruses that cause the 30% of common colds in humans; SARS-CoV, severe acute respiratory syndrome; IBV, infectious bronchitis virus; TCV, turkey coronavirus; FIPV, feline infectious peritonitis virus; FECV, feline enteric coronavirus; CCV, canine coronavirus; TGEV, porcine transmissible gastroenteritis virus; HEV, porcine hemagglutinating encephalomyelitis virus; BCV, bovine coronavirus; RbEVC, rabbit coronavirus; SADV sialodacryadenitis virus; MHV, mouse hepatitis virus. SARS-CoV has been detected in kidneys from infected patients; however, the clinical implications remain unknown.

included in the Nidovirales order that also includes the *Arteviridae* and *Roniviridae* families. Whereas *Roniviridae* comprises a new genera of invertebrate viruses (16), some members of the *Arteviridae* family, cause important economic loss in the swine and equine industries (21,80).

Virion structure. Coronaviruses are 60–200 nm round, somewhat pleiomorphic, particles containing an internal helical RNA-protein nucleocapsid surrounded by an envelope containing glycoprotein peplomers (15) (Fig. 1). The coronavirus genome is a very long single stranded, positive sense, polyadenylated RNA of 27–32 kb (39,40,88) (Fig. 2). The virions of all coronaviruses contains the following structural proteins: nucleocapsid (N) protein, a phosphoprotein (377–454 amino acids) that is complexed with genome RNA; M, a transmembrane glycoprotein (225–262 amino acids); S (spike), the highly glycosylated peplomer glycoprotein (1173–1452 amino acids) mediates attachment to cells and induction of fusion with the host cell membrane during entry for all coronaviruses and the induction of cell to cell fusion during infection for some group 2 some coronaviruses (74,90); E, a membrane associated protein (with a range of 76–108 amino acids) that plays a role in assembly of virions (107). Some group 2 viruses including OC43 and some strains of MHV, express a fifth structural protein, the hemagglutinin-esterase protein (HE), which forms smaller spikes on virions (59); its function in the virus life cycle is as yet unknown. An HE protein is not encoded in the SARS-CoV genome (81).

Replication. During lytic infection of cultured mouse cells, the virus enters the cell via attachment of S protein to a receptor which has been identified as a carcinoembryonic antigen (CEACAM) for MHV (23,24), and aminopeptidase N (APN or CD13) for the group 1 viruses such as 229E and transmissible gastroenteritis virus (TGEV), feline infectious peritonitis virus (FIPV) and canine coronavirus (CCV) (93,94). Receptor interaction triggers fusion of the viral and plasma membranes allowing entry of the nucleocapsid into the cytoplasm (61,108). Virus-specific RNA and proteins are synthesized probably entirely in the cytoplasm (57,101) (Fig. 3). The 5′ two thirds of the genome encode the replicase gene (approximately 21 kilobases) which is expressed via two very large ORFs, 1a and 1b. Expression of coronavirus proteins starts with translation of two polyproteins, pp1a and pp1ab, with predicted lengths of approx 4000 and 7000 amino acids, respectively. The latter is the result of a translational frameshifting event at the end of ORF1a (8). These polyproteins undergo co-translational proteolytic processing into at least four key enzymes: an RNA-dependent RNA polymerase (RdRp), a picornavirus 3C-like proteinase (3CLpro), a papain-like proteinase

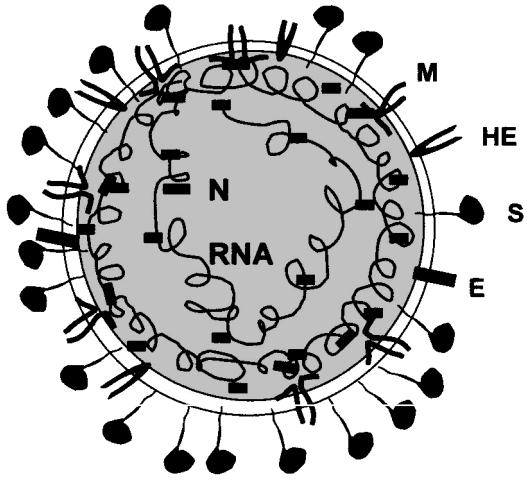

FIG. 1. Model of coronavirus virion structure. Viral particles contain an internal helical RNA-protein nucleocapsid surrounded by an envelope containing glycoprotein peplomers. Nucleocapsid (N) protein is a phosphoprotein that is complexed with genome RNA. S, spike glycoprotein, forms the large glycosylated peplomers that are characteristic of coronaviruses. M, transmembrane protein, is highly hydrophobic and spans the membrane three times. E, a membrane associated protein, is a minor component of the membrane. Some group 2 viruses including OC43 and some strains of MHV, express a fifth structural protein, the hemagglutinin-esterase protein (HE) which forms smaller spikes on virions.

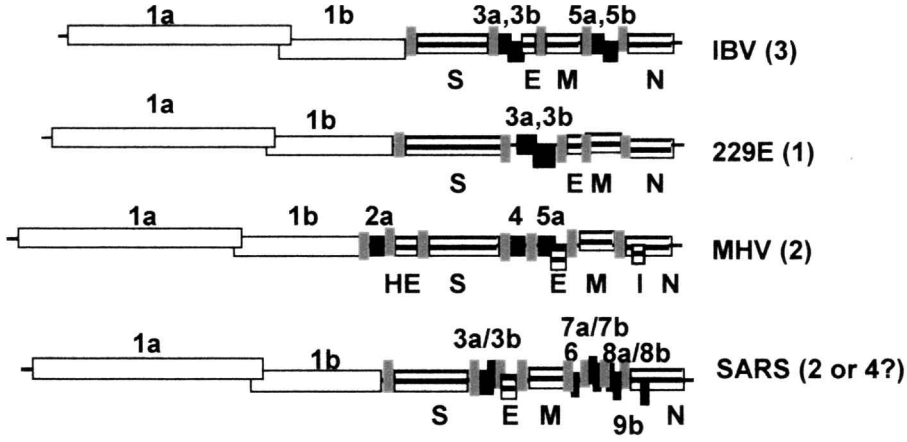

FIG. 2. Coronavirus genomes representing three (or 4?) antigenic groups. Infectious bronchitis virus (IBV, group 3), human coronavirus 229E (group 1) and murine coronavirus (MHV, group 2) are shown along with the SARS-CoV genome. The replicase gene (ORF 1a, 1b) is shown by open bars; structural genes (S, E, M, N and HE) are depicted with striped bars; non-structural genes (black bars) are variable in number and location in the coronavirus genome among the different viral groups. Small open reading frames (ORFs) are depicted in solid bars.

(PLP), and a helicase (38). The replicase complex is used to transcribe a 3′-coterminal set of nested subgenomic mRNAs, as well as genomic RNA, that have a common 5′ "leader" sequence derived from the 5′ end of the genome. While the actual mechanism of synthesis of mRNAs is not well understood, it is currently believed that subgenomic negative strand mRNAs serve as templates for mRNA (7). The replicase carries out "discontinuous transcription" in the fusion of body and leader sequences in subgenomic RNAs

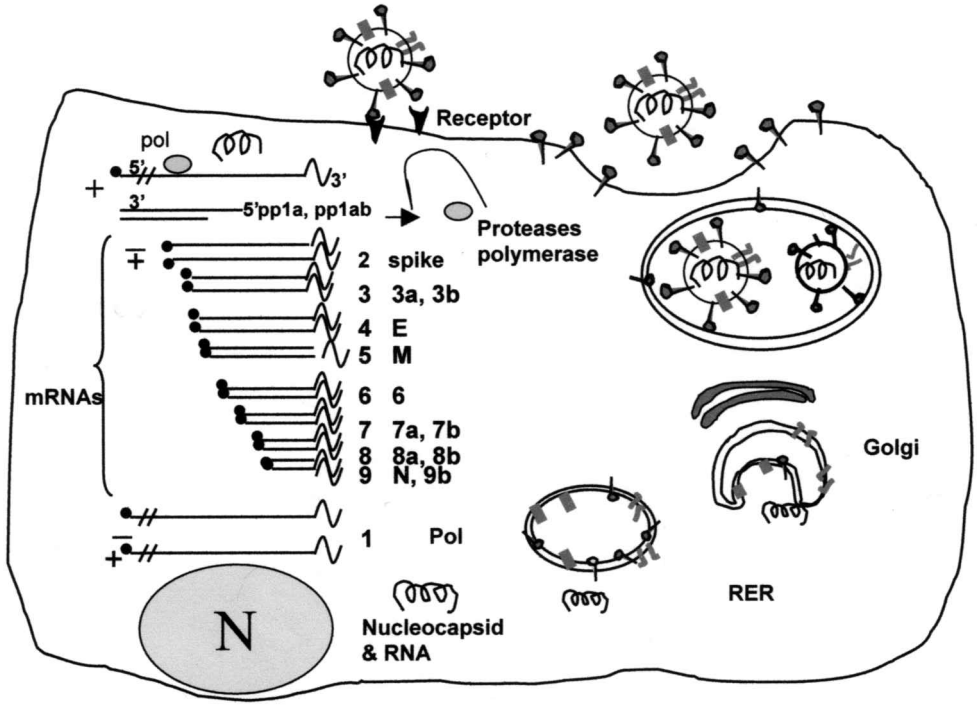

FIG. 3. Model of coronavirus replication. The virus life cycle is described in the text. Polymerase (pol); rough endoplasmic reticulum (RER); nucleus (N). Polyprotein 1a,1b (pp 1a.1b).

and also during recombination events which occur at high frequency during coronavirus replication. In the case of some coronaviruses, such as MHV, syncytia are formed by fusion from within, mediated by S protein on the plasma membrane. New virions are assembled by budding into intracellular membranes and are released from the cells probably through vesicles by the cell secretory mechanisms. Cellular syncytia eventually lift off the tissue culture plastic and the cells die. In addition to lytic infection, some coronaviruses may cause persistent infection in cells in vitro as well as in animals (56,89). In the case of non-fusogenic viruses such as SARS-CoV there is lytic destruction of the cells (50).

Pathogenesis. Coronaviruses infect many species of animals including humans. While human coronaviruses 229E and OC43 cause 30% of colds (46); other coronaviruses infect other organ systems, such as the gastrointestinal track and central nervous system, causing more severe and sometimes economically important diseases in pigs, cows, chickens as well as in laboratory mice. Coronavirus infections can sometimes cause fatal diseases; these include FIPV of the cat, hemagglutinating encephalomyelitis (HEV) of the pig, infectious bronchitis virus (IBV) in the chicken and MHV in the mouse. MHV infection of the mouse, using the commonly studied strains of A59 and JHM, provides a convenient small animal model for the study of acute hepatitis and encephalitis, as well as for chronic demyelinating disease such as multiple sclerosis (42). In immune competent animals, infection with coronaviruses induces a protective immune response including cell mediated immunity and antibodies. However the immune response may also contribute to disease, at least in the case of MHV (79) and FIVP (13). The possibility of immunopathology must be considered in designing vaccine strategies for SARS-CoV.

As a general rule (with some exceptions), coronaviruses are limited to replication in cells of one species. In the case of MHV, only murine cells lines may be productively infected. However when mammalian cells are transfected to express the receptor CEACAMI, they do become permissive for replication of MHV (24). Likewise, the human 229E virus can infect cells of other species when transfected with the human APN receptor (104). Thus, for coronaviruses, the ability to replicate in a cell type is usually dependent on viral entry and not intracellular events. There have been several studies adapting MHV to replicating in other cells types. Variant MHVs, able to replicate in human and hamster cells and monkey cells for example were isolated either by persistent infection of murine cells or infection of mixed cultures of murine and non permissive hamster cells (4,83). The adaptations required mutations in the spike protein and the selection of viruses able to use the mammalian CEACAMI receptor; furthermore, these mutants retain broader host range, infecting cells of multiple species. These studies concluded that, in persistently infected murine cell lines, there is a strong selective advantage for virus with altered interactions with receptor. This selection is likely driven by the very low levels of expression of viral receptor MHVR and/or expression of alternative virus receptors of lesser efficiency. Furthermore, MHV persistence may promote virus cross-species transmissibility by selecting for virus variants that recognize phylogenetic homologues of the normal receptor.

ROLE OF CORONAVIRUS GENES IN PATHOGENESIS

Spike protein. Coronavirus spike proteins are believed to be a major determinant of pathogenic phenotype. Spike proteins both interact with receptor and contain many of the determinants of immune response and thus are often considered as the first target for coronavirus vaccine development (28,31,77). Much indirect evidence indicated that spike was important in determining pathogenic outcome of infection. Early studies demonstrated a relationship between variations in the spike and changes in viral pathogenesis, such as attenuation of neurovirulence of MHV (9,10,18,36,44). Studies on viral determinants of pathogenesis have been limited to the comparison of naturally occurring strains with mutant viruses. This limitation was mainly due to the lack of reverse genetics systems and infectious molecular clones for coronaviruses, and the inability to carry out reverse genetics, which was in turn largely due to the tremendous size of coronavirus genomes. However, in the last 10 years, a targeted RNA recombination system was developed for MHV by Dr. Paul Masters. This reverse genetics system exploits the high frequency of homologous RNA recombination of coronaviruses and allows the introduction of site directed mutations within most of the genes within the coronavirus genome (60). Targeted RNA recombination has been extended to other coro-

navirus systems and has been used to directly demonstrate that the spike is a major determinant of pathogenic phenotype for FIPV (42) as well as MHV (64,65,78) and IBV (11). Of note, infectious clones have only been developed very recently for TGEV (2,91,105), MHV (106), and HCoV-229 (91) coronaviruses, using various strategies.

There are no crystal structures available for any coronavirus spikes as yet; the model depicted in Figure 4 is based on other viral attachment/fusion proteins, and shows a diagram of the murine coronavirus spike protein. Figure 4A shows a model of the structure of the trimerized spike on the viral and/or cell membrane, while Figure 4B shows a linear map of the protein. Spike is synthesized as a 120-kDa precursor, which is co-translationally glycosylated to a 180-kDa polypeptide, which is cleaved intracellularly into two approximately 90-kDa non-covalently associated subunits, S1 and S2. S2 is anchored in the viral or cell membrane, while S1 is modeled as the globular head. S1 contains the receptor binding domain (RBD) as well as a hypervariable domain (HVR) while S2 is highly conserved, containing features common to many viral fusion proteins, including two heptad repeat domains (HR1 and HR2) as well as a transmembrane domain (TM) (5,34,51,71). These domains are believed to be important in viral entry and in the cell-to-cell fusion process (35). In the case of the murine coronavirus, MHV strain JHM, for example, the spike protein contains most of the (H-2^b) CD4 (up arrows) and $CD8^+$ T (down arrows) cell epitopes (75). However, the immunodominant $CD4^+$ epitope is in the membrane protein (103). Spike is also a major target of neutralizing antibodies. There are B cell epitopes in both S1 and S2; the major neutralizing epitope, however, are within S1 (12,76). Thus, S1 is the more variable portion of the spike and contains most of the targets for B and T cells. By sequence comparison of the SARS-CoV spike with the already described and sequenced coronavirus spike proteins, it appears that S2 is more conserved than S1, and that the SARS-CoV spike has the two heptad repeats as well as TM domain (see Fig. 1C). The sequence of the SARS-CoV spike suggests that it is not cleaved in that the SARS-CoV spike lacks the BBXBB (B = basic residue) sequence believed to be the recognition site for cleavage by furin like enzymes. This lack of cleavage is probably the reason for the inability to induce cell to cell fusion as for the known coronaviruses, cleavage appears to be prerequisite for the induction of efficient cell to cell fusion. The S1 domains, among the known coronaviruses, share much less homology than S2 domains and there is presently no way to predict location of the SARS-spike receptor interacting domain and to determine whether there is hypervariable domain, which in the murine coronavirus contains determinants of virulence. The understanding of the SARS-CoV spike gene is important in terms of vaccine design.

Small sequence changes, as little as one amino acid substitution within the spike protein can vastly effect pathogenesis; for example, one amino acid change in the amino terminus of spike of MHV strain A59

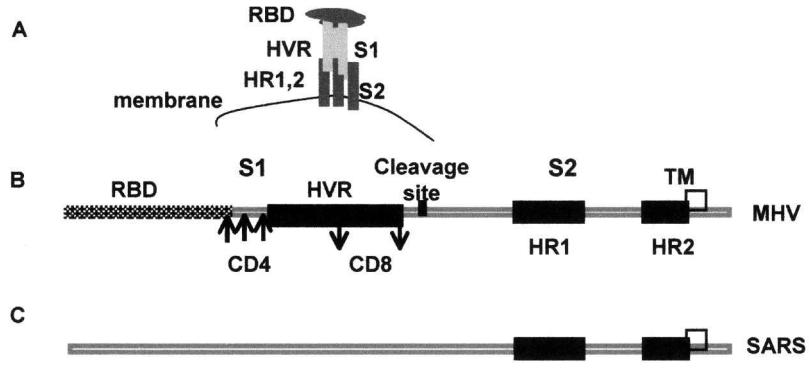

FIG. 4. Coronavirus spike proteins. (**A**) A model of a murine coronavirus (MHV) spike in the membrane. (**B**) A linear diagram of the MHV (strain JHM) and its functional domains. S1 and S2 are the two non-covalently linked subunits. The cleavage site is shown as are the $CD4^+$ and $CD8^+$ (H-2^b) T cell epitopes. RBD (receptor binding domain); HVR (hypervariable domain); heptad repeat domains (HR); TM (transmembrane domain) are shown. (**C**) A diagram of the SARS protein. The HR and TM domains are predicted to be conserved among coronavirus spikes, including SARS-CoV.

(Q159L) can abrogate hepatotropism while retaining virulence in the central nervous system (45,58). A one amino acid substitution within the spike protein of MHV, JHM strain, can highly attenuate and restrict viral antigen to the olfactory bulbs of infected mice (72,95) . Consequently, changes in SARS-CoV spike are expected to have a key role in pathogenesis.

Although the targeted RNA recombination is a powerful technique to manipulate coronavirus genome, and its application has unequivocally demonstrate the role of the spike gene in coronavirus host tropism and pathogenesis, there is still a challenge, that is to understand in mechanistic terms the role of coronavirus spikes in viral entry and pathogenesis. To achieve this goal, other approaches should be considered such as the generation and use of viral pseudotypes. Viral pseudotyping has been exploited for the molecular analysis of envelope glycoproteins of HIV, Ebola, and hepatitis C viruses, among others (26,48,53,102). To our knowledge, there have not yet been any published reports of the application of this technology to coronavirus spike proteins. We have recently been able to incorporate MHV spike proteins into HIV particles. As illustrated in Figure 5, such pseudotypes enter murine L2 and 17.CL-1 cells, but not human (293T cells) or green monkey kidney cells (Vero E6) (Navas and Weiss, manuscript in preparation) (Fig. 5). This methodology will allow the precise investigation of coronavirus spikes in terms of receptors, inhibitors of entry, host-range, and neutralization by antibodies.

Genes other than the spike, so-called "background genes," also influence pathogenesis. Interestingly, a recombinant virus expressing the spike gene of the hepatropic A59 strain of MHV within the background of the neurotropic JHM strain, is very poorly hepatotropic. Thus, the JHM genetic background, plays a dominant role over the spike in the determination of hepatotropism (66).

M protein. The coronavirus M protein is the most abundant envelope component. While group 1 and group 3 coronaviruses have M proteins with N-linked sugars, the M proteins of the group 2 coronaviruses [e.g., mouse hepatitis virus (MHV)] are O-glycosylated. It is not clear whether there is biological significance associated with the difference in glycosylation among the M proteins of the groups of coronaviruses. Although glycosylation is not required for viral assembly, its role in pathogenesis remains unknown. For the porcine coronavirus, TGEV, mutations in the M protein ectodomain that impair *N*-glycosylation, decrease the interferogenic activity (54), and antibodies directed to the TGEV M ectodomain block IFNα induction (14). For MHV, recombinant viruses expressing *N*-, *O*-, or unglycoysylated M proteins were selected and compared (19). The M protein glycosylation state did not influence the tissue culture growth characteristics of the recombinant viruses. However, in mice, the recombinant virus differed in their ability to replicate in the liver, but not in the brain. In tissue culture, the recombinant virus expressing the *N*-glycosylated M protein replicated to higher titer than that expressing the *O*-glycosylated M , which in turn replicated to higher titer than the virus expressing an unglycosylated M protein. Curiously while, the ability to induce interferon *in vivo* did not appear to be affected by their glycosylation status, the ability to replicate in the liver correlated with the ability to induce interferon *in vitro.*

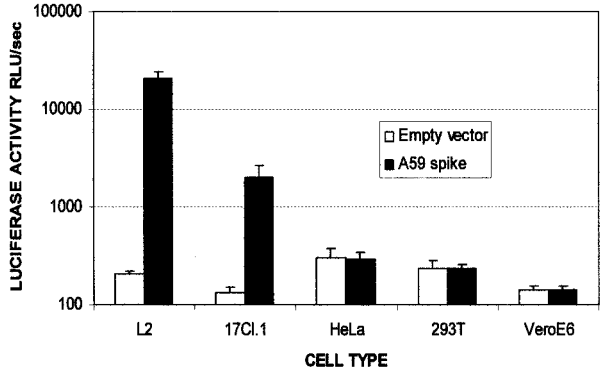

FIG. 5. Infectivity of MHV-A59 spike-pseudotyped HIV particles in murine fibroblast cells (L2 and 17.CL-1), human cells (HeLa, and 293T cells), and African green monkey kidney cells (Vero E6). Pseudotype infectivity correlates with MHV tropism.

N protein. The N gene has been implicated in MHV induced fulminant hepatitis (67,68) In particular, Ding et al. (22) demonstrated that the nucleocapsid protein of MHV-3 strain induces transcriptional activation of a novel prothrombinase gene (fgl2), which is implicated in the coagulation cascade; however, the mechanism remains poorly understood. The expression of fgl2 prothrombinase is restricted to macrophages (Kuppfer cells) and endothelial cells in the liver, but not in hepatocytes. The possible relevance of these findings to SARS disease needs to be further evaluated, as the presence of vascular thrombosis has been reported in some postmortem lung tissues from SARS patients (73).

The genomes of several group 2 coronaviruses, including mouse hepatitis virus (MHV), contain an internal open reading frame (ORF) within the 5′ half of the nucleocapsid gene. This ORF, translated in the +1 reading frame with respect to the N protein, encodes a mostly hydrophobic 23-kDa polypeptide. In the case of MHV, the I gene product is expressed in MHV-infected cells and found within the virions as well. Selection and characterization of recombinant viruses in which the I gene is disrupted demonstrated that I protein is not essential for the replication of MHV either in tissue culture or for pathogenesis in the mouse (32). However, it may have an as yet unknown, subtle role in pathogenesis.

Envelope (E) protein. The E protein is an integral membrane protein and a minor component of the coronavirus envelope (107). E plays an important role in coronavirus assembly (97). Expression of the E protein alone results in its incorporation into vesicles that are released from cells, and the coexpression of the E protein with the membrane protein M leads to the assembly of coronavirus-like particles. *In vitro* studies have demonstrated that E induces apoptosis in MHV-A59–infected 17Cl-1 cells via a caspase dependent mechanism (3). Recently reverse genetics techniques were used to select recombinant MHVs in which the E gene was deleted (along with ORFS 4 and 5a) (52). Surprisingly such viruses were viable demonstrating that E protein is not essential for replication *in vitro*. However, these viruses had small plaque size, low growth rate, and low infectious titer providing further support for the importance of E protein in MHV replication; however, the role of the E gene is pathogenesis beyond affecting replication rate has not been explored *in vivo*.

Small open reading frames (ORFs). All coronaviruses, including SARS-CoV, encode, in addition to structural proteins and replicase proteins, small unique likely nonstructural proteins of unknown functions. In the case of MHV, these are ORFS 2a, 4, and 5a; the proteins encoded in these three ORFs appear or be non essential for replication. In some strains of MHV, ORF 4 is interrupted and becomes ORFs 4a and 4b (100). There is a report of an MHV isolate with a deletion of ORF2a (84). A recombinant MHV lacking gene 4 has been shown to be as virulent as wild type in mice (69). There is some evidence that the protein encoded in these small ORFs may have functions in pathogenesis. While, a recombinant MHV lacking ORFs 2a, 4, and 5 replicates almost as efficiently as wild type virus in tissue culture, it is highly attenuated *in vivo* (20). Furthermore gene 7 of the porcine coronavirus TGEV is non-essential for replication but does influence *in vivo* replication and virulence (70). The SARS coronavirus genome sequence predicts 8 such ORFs (ORFs 3a, 3b, 6, 7a,7b, 8a, 8b, and 9b) (87). While there are not yet any specific examples of coronavirus proteins involved in anti-host defense there are many examples of non-essential proteins that have such functions in other viral systems of such proteins. The HIV Vif protein, which is dispensable in cell types yet essential, is some cell such as primary T cells, is believed to overcome an antiretroviral activity of the innate immune response that is mediated by deamination of viral DNA (43). The Herpes simplex proteins ICP47 blocks the loading of peptides onto MHC class I and subsequent cell surface expression of the peptide/MHC complex (25). The nonstructural (NS) gene of the highly lethal H5N1influenza viruses, unlike other human, avian and swine influenza viruses, confers resistance to the antiviral effects of interferons and tumor necrosis factor alpha (85).

Replicase. Lastly the replicase proteins, and perhaps other non structural proteins, could affect tropism and pathogenesis by determining rate of replication of virus perhaps by interactions with cell type specific factors or with elements of the immune response. This hypothesis needs to be further evaluated in the future.

PERSPECTIVES: CORONAVIRUS AS EMERGING PATHOGENS

The origins of SARS-CoV are not known. The genome does not appear to be recombinant of any known coronaviruses. One prominent theory is that SARS-CoV has a reservoir in another species and "jumped"

to humans (30). This has been given credibility by the isolation of SARS like coronavirus from several species of wild animals (six civet cats among them) (30) in markets of Southern China and the report that pet cats were found to be infected and sick from the SARS-CoV in the Amboy Garden apartment complex in Hong Kong, location of more than 100 human SARS in infections (1,17). It has recently been reported that the genomes of viruses isolated from civet cats are close in sequence to the human isolates; there are differences in the spike gene and in one of the small, likely nonstructural, open reading frames (41). A recent comparison of the genomes of human and animal isolates of SARS-CoV indicates ten consistent amino acid differences between the spike proteins of human and animal isolates (41); interestingly, these substitutions suggest the loss of multiple predicted glycosylation sites in the spikes of the animal isolates. This is a potentially exciting finding and may indicate a difference in biological properties. The loss of glycosylation sites in the glycoprotein of mutant simian immunodeficiency virus has been shown to increase neutralization of these viruses (49). Among the differences between the animal and human isolates, there are also two amino acid substitutions within the heptad repeat domains regions of the spike; these domains are involved in membrane fusion and aa substitutions are known in fusion domains to effect pathogenic properties in the murine coronavirus (72,95). The other consistent difference observed in the comparison of the genomes of SARS-CoV isolates from humans and civet cats, was a 29-nt insertion in the genomes of animal isolates. These results in the merging of ORFs 8a and 8b so there is only ORF8 (87). In another report, an in frame deletion of 45 nt occurs in ORF 7b after three passages of SARS CoV in tissue culture (92). These small ORFs may play a role in biological properties of SARS-CoV isolates and should be explored further.

Thus, at this point, it not clear what the natural host of the SARS-CoV is and how it "jumped" into the human population; it will be important to understand more about the sequence differences in the spike proteins between animal and human isolates and how the spike protein controls species tropism. The knowledge accumulated by the study of the coronaviruses has already accelerated the understanding of the SARS coronavirus and will continue to do so in the future.

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Address reprint requests to:
Susan R. Weiss, Ph.D.
Department of Microbiology
University of Pennsylvania School of Medicine
36^{th} Street and Hamilton Walk
Philadelphia, PA 19104-6076

E-mail: weisssr@mail.med.upenn.edu