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Review

Coronavirus replication and pathogenesis: Implications for the recent outbreak of severe acute respiratory syndrome (SARS), and the challenge for vaccine development

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A novel coronavirus has been recently identified as the causative agent of the severe acute respiratory syndrome (SARS) outbreak that has accounted for more than 8000 infected people worldwide. This review will discuss current knowledge on coronavirus replication, pathogenesis, evolution, and vaccine strategies, as well as the most recent findings on SARS coronavirus. *Journal of NeuroVirology* (2004) **10,** 75–85.

Keywords: coronavirus mutation; replication; SARS; vaccine

Coronaviruses and the outbreak of SARS

Coronaviruses comprise a large group of enveloped, positive-sense, single-stranded polyadenylated RNA viruses classified in the Nidovirales order (Gonzalez *et al*, 2003; Siddell, 1995). Coronaviruses have the largest viral RNA genomes known (ranging from 27.6 to 31.6 kb). Coronaviruses are classified into three groups (I to III) based on serological cross-reactivity (Enjuanes, 2000) (Table 1). Recently, phylogenetic analysis studies have supported the existence of these three groups (Gonzalez *et al*, 2003). Group I coronaviruses include important animal pathogens, such as the porcine transmissible gastroenteritis virus (TGEV), porcine epidemic diarrhea virus (PEDV), and feline infectious peritonitis virus (FIPV). Group II also includes pathogens of veterinary relevance, such as the bovine coronavirus (BCoV), porcine hemagglutinating encephalomyelitis virus (HEV), and equine coronavirus (ECoV). Murine hepatitis virus (MHV) and rat coronavirus (RtCoV) also belong to group II. Group III coronaviruses infect avian species, and at the present time only three viruses have been assigned to this group, infectious bronchitis virus (IBV), turkey coronavirus (TCoV), and pheasant coronavirus (Cavanagh *et al*, 2002). Human coronaviruses belongs to groups I (HCoV-229E) and II (HCoV-OC43) (discussed below).

Coronaviruses can now be considered as emerging pathogens. Although the first cases of "an atypical pneuomonia" appear to have originated in Guangdong Providence (China) in late 2002 (Parry, 2003), it was not until February 2003 that the World Health Organization (WHO) received reports from China of an outbreak with more than 300 cases and 5 deaths in Guangdong Providence (WHO, 2003). Late in February 2003, the epidemic spread from Guangdong to a hotel in Hong Kong by a doctor from Guangdong Providence who was treating atypical pneumonia patients (Lee *et al*, 2003). The epidemic appears to have spread from this hotel in Hong Kong to Hanoi by an Chinese-American business man who had been in that hotel. By then, Dr. Carlo Urbani, who was a WHO officer based in Hanoi, was the first to identify the symptoms of this atypical pneumonia with a new respiratory illness, which he called "severe acute respiratory syndrome" (SARS). Dr. Urbani died on March 29 in Bangkok with symptoms of the new disease (Reilley *et al*, 2003). By late March, SARS had spread to many locations, mostly in Asia, but also to Toronto, Canada (Poutanen *et al*, 2003). A novel virus was isolated from patients' lungs and sputum

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Table 1 Coronavirus groups, host tropism, receptors, and diseases

Host	*Group*	*Virus*[a]	*Receptor*	*Co-Receptor*	*Disease*[b]
Cat	1	FIPV	Feline APN		R, E, S
Cat	1	FCoV	Feline APN		E
Dog	1	CCoV	Canine APN		R, E
Pig	1	TGEV	Porcine APN		(R) E
Pig	1	PRCoV	Porcine APN		R
Pig	1	PEDV	Porcine APN		E
Human	1	HCoV-229E	Human APN		R
Human	?	SARS-CoV	ACE2		R, E
Human	2	HCoV-OC43	?	9-0AcNA	R
Mouse	2	MHV	mCEACAM1		(R) E, CNS
Rat	2	RCoV	?	9-0AcNA	R
Pig	2	HEV	?	9-0AcNA	E
Cattle	2	BCoV	?	9-0AcNA	R, E
Chicken	3	IBV	?		R, E
Turkey	3	TCoV	?		E
Pheaasant	3	PHCoV	?		R, nephritis

[a]HCoV-OC43 and HCoV-OC229 cause the 30% of common colds in humans; SARS-CoV, severe acute respiratory syndrome; FIPV, feline infectious peritonitis virus; FCoV, feline enteric coronavirus; CCV, canine coronavirus; TGEV, porcine transmissible gastroenteritis virus; PEDV, porcine epidemic diarrhea virus; MHV, mouse hepatitis virus; RCoV, rat coronavirus; HEV, porcine hemagglutinating encephalomyelitis virus; BCoV, bovine coronavirus; IBV, infectious bronchitis virus; TCV, turkey coronavirus; PHCoV, pheasant coronavirus.
[b]R, respiratory; E, enteric; S, systemic; CNS, central nervous system.

and cultivated in a monkey kidney cell line (Vero E6) (Drosten *et al*, 2003a, 2003b; Ksiazek *et al*, 2003; Peiris *et al*, 2003b). The SARS epidemic was officially controlled by July 2003 (Ashraf, 2003; Fleck, 2003). Strikingly, the epidemic was controlled by isolation only. The worldwide SARS epidemic has accounted for more than 8000 infected people worldwide and more than 800 deaths, with mortality rates that vary and are somewhat dependent on age (5% to 43%) (Donnelly *et al*, 2003).

SARS infection exhibits a wide clinical course, mainly characterized by fever, dyspnea, lymphopenia, and lower tract respiratory infection (Nie *et al*, 2003; Tsui *et al*, 2003). Concurrent gastrointestinal symptoms and diarrhea have also been reported (Booth *et al*, 2003; Lee *et al*, 2003; Leung *et al*, 2003; Peiris *et al*, 2003a). Although the route of transmission has not been clearly established, airborne droplets from infected patients may be the main route of transmission (Zhong *et al*, 2003). However, blood transmission and fecal-oral transmission cannot be rule out. Strikingly, it has been recently reported that the SARS coronavirus (SARS-CoV) replicates in peripheral blood mononuclear cells (PBMCs) from SARS patients (Li *et al*, 2003a). Furthermore, active replication of SARS-CoV in both the small and large intestine has been recently demonstrated. In addition, SARS-CoV was detected in the stools of the patients for more than 10 weeks after symptom onset (Leung *et al*, 2003).

The mechanism of injury caused by SARS-CoV infection remains unknown. A SARS disease model has been proposed, consisting of three phases: viral replication, immune hyperactivity, and pulmonary destruction (Tsui *et al*, 2003). SARS pathology of the lung has been associated with diffuse alveolar damage, epithelial cell proliferation, and an increase of macrophages. Multinucleate giant-cell infiltrates of macrophage or epithelial origin have been associated with putative syncytium-like formation that is characteristic of many coronavirus infections (Nicholls *et al*, 2003). Lymphopenia, hemophagocytosis in the lung, and white-pulp atrophy of the spleen observed in SARS patients are reminiscent of that reported in fatal influenza subtype H5N1 disease in 1997 (To *et al*, 2001). Strikingly, the presence of hemophagocytosis supports a cytokine dysregulation (Fisman, 2000). Proinflammatory cytokines released by stimulated macrophages in the alveoli may have a role in the pathogenesis of SARS. Based on this cytokine deregulation hypothesis, treatment of SARS-infected patients has included the administration of steroids, aimed to modulate the exacerbated cytokine response, similarly to the treatment of nonviral acute respiratory distress syndrome (Lai *et al*, 2003). However, treatments of SARS infection have been ineffective (Koren *et al*, 2003; Lee *et al*, 2003; Tsui *et al*, 2003). Treatment have been based in the administration of antibacterials (to prevent secondary bacterial infections) and steroids (methylprednisolone, or corticosteroids, to modulate cytokine dysregulation) in combination with ribavirin, a nucleoside analog with broad antiviral activity that is being used for the treatment of respiratory syncytial virus (RSV) infection (Everard *et al*, 2001) and for the management of hepatitis C infection (Lipman and Cotler, 2003; Martin *et al*, 1998). At the present, the lack of ribavirin response is not understood. We have recently proposed a possible mechanism for the natural resistance of SARS-CoV to ribavirin based on a molecular model of SARS-CoV polymerase (Xu *et al*, 2003). We propose that the clinically observed resistance of SARS to ribavirin is probably due to perturbation of a conserved motif A that controls rNTP binding and fidelity

of polymerization (Xu *et al*, 2003). Recently, the antiviral potential of interferons (IFNs) α, β, and γ has been assessed in Vero and Caco2 cell cultures, IFN β being the most potent inhibitor of SARS-CoV (Cinatl *et al*, 2003).

Coronaviruses induce acute self-limited as well as chronic persistent infections, and cause a wide range of pathologies, such as acute respiratory disease, encephalitis, chronic demyelination in the central nervous system (CNS), hepatitis, and enteritis (Holmes, 1996). In general, coronaviruses infect the respiratory and enteric mucosal surfaces, although macrophages, hepatocytes, endothelial cells, neurons, oligodendrocytes, and astrocytes are main target cells for some coronaviruses, such as MHV (Haring and Perlman, 2001; Navas-Martín and Weiss, 2003). Some coronaviruses have an important impact on farm animals, i.e., porcine (TGEV), avian (IBV), and bovine (BCoV) coronaviruses causes respiratory and enteric infections that account for severe economic loss (Holmes, 1996). In addition, coronaviruses are also being studied as animal models for viral pathogenesis. For example, some strains of murine coronavirus (MHV) cause encephalitis and chronic demyelination in experimentally infected mice, thus MHV infection of the mouse is used as an animal model for human CNS demyelinating diseases such as multiple sclerosis (Buchmeier and Lane, 1999; Haring and Perlman, 2001).

Before the discovery of a previously unknown coronavirus as the causative agent of the SARS, two coronaviruses were known to infect humans, causing self-limiting upper respiratory tract infections (Myint, 1994). These human coronaviruses, HCoV-229E and HCoV-OC43, cause about the 30% of the common colds and have never been reported to cause severe illness. However, human coronaviruses have been reported to be associated with multiple sclerosis, although this has never been confirmed (Talbot, 1995). So far, the more direct evidence that could suggest a neurotropic potential is the fact that both HCoV-229E and HCoV-OC43 can infect primary cultures of human neural cells, in particular fetal astrocytes (Bonavia *et al*, 1997). Strikingly, SARS-CoV has been detected by real-time polymerase chain reaction (PCR) in the cerebrospinal fluid of a patient with SARS, who also showed an epilepticus status (Hung *et al*, 2003). The significance of this finding is limited because no other association with CNS symptoms in SARS-infected patients have been reported so far.

Although the origin of the SARS-CoV remains unknown, phylogenetic analysis has demonstrated that SARS-CoV belongs to the Coronaviridae family (Marra *et al*, 2003; Rota *et al*, 2003). SARS-CoV genome organization shares some hallmarks of other coronavirus genomes, although some characteristics are unique to SARS-CoV (Figure 1). For example, unlike group II coronaviruses, SARS has no hemagglutinin (HA) esterase (HE) gene, a gene homologous to the HA from influenza C virus (Luytjes *et al*, 1988).

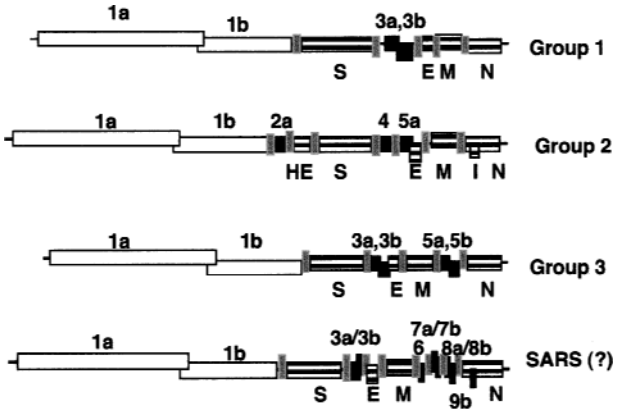

Figure 1 Coronavirus genomes representing three antigenic groups. SARS-CoV has been suggested to define either a new coronavirus group (4), an early spilt-off from group 2, and a recombinant virus (discussed in the text). Genome organization of coronavirus groups 1, 2, and 3 are shown along with the SARS-CoV genome. The replicase gene (ORFs 1a, 1b) is shown by open bars; structural genes (S, E, M, N, and HE) are depicted with striped bars; nonstructural 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.

Like group I coronaviruses, SARS-CoV spike protein lacks a cleavage recognition site. Like group III coronaviruses, SARS-CoV has only one papain-like protease (plp-2) encoded in open reading frame 1a. Similar to group III, at the 3′ UTR region of SARS, there is a conserved sequence motif that is though to have been acquired from astroviruses (Jonassen *et al*, 1998). Early after its discovery, SARS-CoV was suggested to define a new group (IV) within the coronavirus genus (Ksiazek *et al*, 2003; Marra *et al*, 2003; Rota *et al*, 2003). However, other report has suggested that it is more directly related to group II coronaviruses, along with the bovine coronavirus, the human OC43 virus, and the murine coronavirus (MHV) (Snijder *et al*, 2003). Rest *et al* have recently suggested that the SARS-CoV polymerase may be a result of recombination (Rest and Mindell, 2003). Based on this rooted analysis using Bayesian inference (Huelsenbeck *et al*, 2001), the 5′ RNA-dependent RNA polymerase (RDRP) fragment diverges from others coronaviruses prior to divergences between and within groups I to III, whereas there is a sister relationship between the more recently diverged SARS-CoV 3′ RDRP polymerase fragment and group 3 avian coronaviruses (Rest and Mindell, 2003). It is worth noting, however, that these authors have used Bayesian inference and amino acid sequences rather than nucleotides in their phylogenetic analysis.

The molecular determinants that may account for the dramatic differences in pathogenesis between the human coronaviruses (HCoV-229E, HCoV-OC43) and SARS-CoV are currently unknown and are almost certainly multigenic. Although coronaviruses infect a broad range of animals, including humans, other

mammals, and birds, it has been accepted that each individual virus has very restricted host range. This is based on the observations that, thus far, the ability of coronaviruses to replicate in a particular cell type depends solely on the ability to interact with their receptors (Holmes, 1996). For example, murine coronavirus replicates in murine cells, and not in hamster, human, monkey, or cat cells; however, once the nonpermissive cells are transfected with the cDNA encoding MHV receptor, carcinoembryonic antigen-cell adhesion molecule 1 (CEACAM1), they become susceptible to MHV infection (Dveksler *et al*, 1996). This narrow host range is determined by the specific interaction between the spike (S) protein of coronaviruses (present in the viral envelope) and their respective receptor in the cellular membrane. Until now, coronaviruses have been poorly studied from the structural biology standpoint, perhaps mainly due to their lack of clinical relevance. Strikingly, there is no structural data on coronavirus spikes. Although some coronavirus receptors have been identified, the lack of structural data for coronavirus spikes has hampered the study of coronavirus entry. Group I coronaviruses, such as human HCoV-229E, use aminopeptidase N (APN; CD13), a zinc-binding protease, of their normal host species as their receptors (Yeager *et al*, 1992) (Table 1). Human aminopeptidase N is found on the cell surface of apical membranes of intestinal, lung, kidney, and epithelial cells, macrophages, and synaptic junctions (Kunz *et al*, 1994; Look *et al*, 1989). Interestingly, HCoV-229E can utilize either human APN or feline APN as a receptor, but cannot use porcine APN (Wentworth and Holmes, 2001a, 2001b). Among group II coronaviruses, only the receptor for the murine coronavirus (MHV) is known. MHV enter the cell after binding members of the pleiotropic family of carcinoembryonic antigen-cell adhesion molecules (CEACAMs; CD66a) (Chen *et al*, 1995, 1997; Dveksler *et al*, 1993; Nedellec *et al*, 1994). CEACAMs are glycoproteins possessing two or four immunoglobulin-like extracellular domains followed by a transmembrane domain and a cytoplasmic tail (Nedellec *et al*, 1994). CEACAMs are involved in the intercellular adhesion and the development of hepatocellular, colorectal, and epithelial tumors (Beauchemin *et al*, 1999), and are expressed primarily on the epithelial and endothelial cells of the respiratory tract, intestines, and other tissues (Godfraind *et al*, 1995; Robitaille *et al*, 1999). Interestingly, they are not well expressed in the brain, a major site of MHV infection. The receptors for the other members of group II coronaviruses remain unknown, although it is known they can use 9-*O*-acetylated sialic acids as coreceptor (through binding to the HE glycoprotein present in the viral envelope of group II coronaviruses (Holmes, 1996). Strikingly, angiotensin-converting enzyme 2 (ACE2) has been recently identified as a functional receptor for SARS-CoV (Li *et al*, 2003b). ACE2 is like APN, a zinc metalloprotease.

Coronavirus biological vectors are not known. However, it has been speculated that SARS-CoV has "jumped" from an animal to humans (Holmes, 2003). It is suspected that SARS-CoV has a reservoir in a wild animal species (Holmes, 2003). A range of domestic and wild mammals in Guangdong Province have been examined in order to identify animals carrying SARS-CoV. Interestingly, SARS-like viruses were isolated from live small, wild mammals (Himalayan palm civets, raccoon dogs, and Chinese ferret badgers) in a retail market in China (Guan *et al*, 2003). Recent data indicate that ferrets and domestic cats are susceptible to SARS-CoV infection and that they can transmit the virus to previously uninfected animals that are housed with them (Martina *et al*, 2003).

Coronavirus replication

Coronavirus RNA synthesis occurs in the cytoplasm via a negative-strand RNA intermediate (Holmes, 1996). The virion RNA is infectious and functions as an mRNA, having a 5′ terminal cap followed by a leader sequence and an untranslated region. At the 3′ end of the genome, there is an untranslated region followed by a poly (A) tail. Coronaviruses have a polycistronic genome organization and synthesize multiple subgenomic mRNAs, all overlapping at the 3′ end (nested set of subgenomic RNAs) and all containing the same 5′ leader sequence derived from the 5′ end of the genome. Each mRNA is translated to generate the protein product of its most 5′ gene, but sometimes is translated into a second, downstream protein as well (Fischer *et al*, 1997). Coronaviruses replicate by a unique discontinous transcription mechanism that is not completely understood. Discontinuous transcription of subgenomic mRNAs is believed to be regulated by transcription regulating sequences (TRSs, also referred to as intergenic sequence) at the 5′ end of each transcriptional unit (La Monica *et al*, 1992). The current model is that discontinuous transcription occurs during the synthesis of subgenomic negative-sense RNAs; this model is supported by data that demonstrate the existence of transcriptionally active, subgenomic-size negative RNA strands containing the antileader sequence (Snijder *et al*, 2003).

Coronavirus genes are arranged in the order 5′-replicase-(HE)-S-E-M-N-3′, with some other genes that have been found not essential both *in vivo* and *in vitro* (de Haan *et al*, 2002a, 2002b, 2003; Holmes, 1996). The virion envelope surrounding the nucleocapsid contains the following structural proteins: S (spike), M (matrix), E (envelope), and, in the case of group II coronaviruses, HE (hemagglutinin-esterase). S protein is a 180-kDa peplomer glycoprotein found on the virion envelope and on the plasma membrane of infected cells; S contains epitopes for viral neutralization and T-cell response, and

is responsible for attachment to the cellular receptor and for both virus-cell fusion during viral entry, and cell-to-cell fusion for some coronaviruses later during infection (Gallagher, 2001). The spike gene contains determinants of tropism and pathogenesis (Navas-Martín and Weiss, 2003). M (matrix) protein is a transmembrane glycoprotein with its carboxy terminus integrated within the virion core; M is believed to play a key role in maintaining the core structure (Escors *et al*, 2001). E (envelope) is a 9.6-kDa polypeptide membrane associated protein that is critical for virion assembly (Vennema *et al*, 1996; Yu *et al*, 1994) . N, a 60-kDa phosphoprotein complexed with the RNA genome to form the nucleocapsid, forms the virion core or nucleocapsid (Baric *et al*, 1988). New virions are assembled by budding into intracellular membranes and are released from the cells through vesicles of the secretory pathway (Holmes, 1996; Prentice and Denison, 2001). The role of coronavirus structural proteins in pathogenesis has been reviewed elsewhere (Navas-Martín and Weiss, 2003).

Coronavirus evolution

Two major forces drive coronavirus evolution: recombination and mutation. Coronaviruses undergo homologous RNA recombination at high frequencies, although the mechanism is not well understood. Signals for RNA polymerase recognition may play a role in coronavirus recombination. Strikingly, recombination has been reported only among coronaviruses of the same group, although intergroup recombination is theoretically possible. For example, intragroup recombination between the feline and canine enteric coronaviruses (Herrewegh *et al*, 1998), between MHV strains (Keck *et al*, 1988a, 1988b), and between strains of IBV (Jia *et al*, 1995; Kottier *et al*, 1995a, 1995b), has been reported. Recombination events among coronaviruses probably results from a polymerase-jumping mechanism during coinfection (Brian and Spaan, 1997; Lai, 1992). Recombination between animal (bovine coronavirus) and human coronaviruses (HCoV-OC43) in cell culture has been recently reported (Wu *et al*, 2003). A high potential for recombination among members of group II coronaviruses has been suggested (Wu *et al*, 2003). Strikingly, Rest *et al* have found evidence of a recombination breakpoint within the polymerase of SARS-CoV (Rest and Mindell, 2003).

It is well known that RNA viruses mutate at rates in the range of 10^{-3} to 10^{-5} base substitutions per nucleotide copied (Domingo and Holland, 1997; Domingo *et al*, 1997; Holland and Domingo, 1998). These values are several orders of magnitude larger than those encountered during replication of DNA viruses, and many orders of magnitude grater than of cellular DNA (Drake, 1991). As a consequence of this high mutation rates, RNA viruses exits as diverse populations composed of ensembles of closely related, nonidentical genomes that are known as viral quasispecies (Domingo *et al*, 2001). The molecular basis of this complexity is the limited copying fidelity exhibited by the viral replicases (Steinhauer *et al*, 1992).

Evolution of coronavirus during persistent infection has been investigated using murine coronaviruses, both *in vitro* and *in vivo* (Adami *et al*, 1995; Fleming *et al*, 1993, 1995; Rowe *et al*, 1997a, 1997b, 1998; Steinhauer *et al*, 1992). Murine coronaviruses comprise several pathogenic strains historically known as mouse hepatitis virus (MHV), although only a few are primarily hepatotropic (Haring and Perlman, 2001; Navas-Martín and Weiss, 2003). MHV causes both persistent and acute self-limited infections (Haring and Perlman, 2001). MHV naturally infects mice via the respiratory and enteric routes. However, in laboratory strains, the outcome of MHV infection is dependent upon the route and dose of inoculation, and host factors such as age, genetic background, immune status, and virus strain (some are neurotropic, some are hepatotropic, and others exhibits both tropisms) (Siddell, 1995). Although the virus is cleared, viral RNA may persist in the CNS for more that 1 year after infection (Fleming *et al*, 1995). MHV recombinants arise during passage in tissue culture as well as in inoculated mice (Rowe *et al*, 1997a, 1997b, 1998). MHV strain A59 from persistently infected murine cells exhibits an extended host range, being able to infect many cells lines of mammalian origin (Baric *et al*, 1999; Chen and Baric, 1996; Schickli *et al*, 1997, 1998). MHV spike deletion variants have been detected in the CNS (brain and spinal cord) from mice persistently infected with JHM, a highly neurovirulent MHV strain (Fleming *et al*, 1995). Mutants of MHV-A59, unable to induce hepatitis, may be selected by persistent infection of glial cells *in vitro* and hepatotropism revertants of these mutants may be selected from these mutants by multiple passage in the mouse liver (Gombold *et al*, 1993; Hingley *et al*, 1994, 1995; Leparc-Goffart *et al*, 1997). In addition, variants able to evade the CD8 cytotoxic lymphocytic (CTL), response (CTL escape mutants) have been identified in a murine model in which suckling mice are infected with the neurotropic MHV-JHM strain (Perlman and Pewe, 1998; Pewe *et al*, 1996, 1999), or mice that are immunized in an epitope-specific manner prior to infection with MHV-A59 (Chua *et al*, 2004).

Coronavirus vaccines

Currently, much effort is being done to develop vaccine strategies against SARS-CoV. The development

of a vaccine against SARS needs to be based on the limited knowledge gained from studies on the immune response in SARS-infected patients, as well as in the coronavirus vaccine strategies that have been developed over the years. Most of the studies have focused on coronavirus infections on farm animals, but less is known on the immune response against human coronavirus HCoV-229E and HCoV-OC43.

In general, both humoral and cellular immune responses are required to protect against coronaviruses. T- and B-cell epitopes have been mapped to various coronavirus proteins. For example, CD4 T-cell epitopes have been identified in the spike (Xue and Perlman, 1997), M (Xue *et al*, 1995), and nucleocapsid (N) (van der Veen, 1996) proteins of MHV, and in the N protein of porcine TGEV (Anton *et al*, 1995) and avian coronaviruses (Boots *et al*, 1991). MHV spike glycoprotein contains all but one of the known virus specific H-2^b–restricted T-cell epitopes (Haring and Perlman, 2001). Various studies have demonstrated the importance of CD4 and CD8 T cells in host defense and viral clearance from the CNS during murine coronavirus infection (Bergmann *et al*, 1998, 2001; Chua *et al*, 2004; Marten *et al*, 2000, 2001; Perlman and Wu, 2001; Wu *et al*, 2000a, 2000b, 2001). The B-cell response has been shown to be necessary to prevent recurrence of viral replication in the CNS after T cell–mediated clearance in the early acute disease (Lin *et al*, 1999; Matthews *et al*, 2001). However, immune response contributes to pathogenesis in some coronavirus infections. For example, humoral immune response to FIPV may contribute to host pathology (Glansbeek *et al*, 2002), and T cells appear to be involved in the induction of CNS inflammation and demyelination observed in MHV-infected mice (Marten *et al*, 2001).

Most of the current knowledge on coronavirus vaccines has been generated by studies aimed at developing vaccine strategies for coronavirus of veterinary relevance, such as avian IBV and porcine TGEV. Live attenuated as well as killed coronavirus vaccines have been evaluated with some success. For IBV, live attenuated vaccine appears to be more effective than killed vaccine (Farsang *et al*, 2002). A killed canine coronavirus vaccine has been also developed (Pratelli *et al*, 2003), and a killed bovine coronavirus vaccine have been proved to be safe and effective (Takamura *et al*, 2002). Live attenuated coronavirus vaccines have been successfully combined with killed IBV (Farsang *et al*, 2002). A recent strategy to generate live attenuated coronavirus takes advantage of the deletion of "group specific genes" that are specific for each of the groups (de Haan *et al*, 2002a; Ortego *et al*, 2003).

Strikingly, epitope-driven approaches have been successfully attempted for IBV and MHV vaccine strategies. Chickens immunized with a DNA plasmid encoding a CTL epitope from IBV were protected from acute viral infection (Wang and Khan, 2000; Wang *et al*, 2002). Plant viruses, such as a hybrid tobacco mosaic virus (TMV) carrying an epitope from the MHV spike protein, have been successfully used to develop immunity against murine coronavirus (MHV) (Koo *et al*, 1999). Intranasal or subcutaneous administration of this hybrid TMV induces parenteral and mucosal immunization, protecting from MHV challenge (Koo *et al*, 1999). Immunization against a CD8+ epitope of MHV using a recombinant *Listeria monocytogenes* vector or the adoptive transfer of epitope specific CD8+ T cells have been effective in reducing MHV-induced acute CNS disease and demyelination (Chua *et al*, 2004; MacNamara and Weiss, unpublished data). Antibodies elicited with a synthetic peptide comprising a B-cell epitope and a T-helper cell determinant can protect mice against an acute fetal MHV infection (Koolen *et al*, 1990).

Some recombinant constructs (recombinant vectored vaccines) have been shown to confer protection against several coronaviruses. A DNA vaccine containing the nucleoprotein of the porcine TGEV has been used to vaccinate against gastroenteritis, inducing both humoral and cellular immune response (Liu *et al*, 2001). A recombinant fowlpox containing the S1 gene of IBV has been produced and was shown to be relatively protective against IBV (Wang *et al*, 2002). A fowlpox virus expressing carboxyl-terminal nucleocapsid protein of IBV has also been developed (Yu *et al*, 2001).

It should be noticed that in some instances, coronavirus vaccines have been shown to induce enhancement of viral disease after vaccination by a mechanism that is not well understood, but is known to be related to the antibody response to spike. In particular, this has been reported for FIPV using different vaccination strategies (Olsen *et al*, 1993; Weiss and Scott, 1981).

The experience gained through all the vaccine strategies generated against some coronaviruses, such as IBV, TGEV, BCV, FIPV, and MHV should be considered for the development of a SARS vaccine.

SARS update: Receptor, infectious clone

Two major advances for the understanding of SARS pathogenesis, host tropism and vaccine development, have been already achieved. (i) A full-length infectious cDNA clone of SARS-CoV has been successfully assembled (Yount *et al*, 2003). (ii) The discovery of a receptor for SARS-CoV. Li *et al* (2003b) have identified a zinc metallopeptidase, angiotensin-converting enzyme 2 (ACE2) as a functional receptor for SARS-CoV.

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