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 belong 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 pneumonia” 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 hospital. 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.
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 ruled 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.

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

| Host | Group | Virusa | Receptor | Co-Receptor | Diseaseb |
|------|-------|--------|----------|-------------|----------|
| 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        |          |
| Mouse| 2     | HCoV-OC43 | ?        | 9-0AcNA     |          |
| Rat  | 2     | RCoV   | ?         | R           |          |
| Pig  | 2     | HEV    | ?         | R           |          |
| Cattle| 2   | BCoV   | ?         | R           |          |
| Chicken| 3   | IBV    | ?         | R, E        |          |
| Turkey | 3    | TCoV   | ?         | E           |          |
| Pheasant | 3 | PHCoV  | ?         |             | R, nephritis |

aHCoV-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; CCoV, 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.

bR, respiratory; E, enteric; S, systemic; CNS, central nervous system.
of polymerization (Xu et al., 2003). Recently, the antiviral potential of interferons (IFNs) \( \alpha \), \( \beta \), and \( \gamma \) has been assessed in Vero and Caco2 cell cultures, IFN \( \beta \) 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 cause 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 (Luypjes et al., 1988).

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 thought 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., 1998; 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 the 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 non-permissive cells are transfected with the cDNA encoding MHV receptor, carinoembryonic 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 discontinuous 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 (hemagglutininesterase). 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 result 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 greater than of cellular DNA (Drake, 1991). As a consequence of this high mutation rates, RNA viruses exits as diverse populations composed of close 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 replicates (Steinhauer et al, 1992).

Evolution of coronavirus during persistent infection has been investigated using murine coronaviruses, both in vitro and on 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 pathogenic strains (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 exhibit 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 neuroviro- lent 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; Leparcq-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-2b-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 carboxy-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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