Emerging Viruses in Human Populations
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Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV)

Tommy R. Tong
Department of Pathology, Princess Margaret Hospital, Laichikok, Kowloon, Hong Kong, China

Severe acute respiratory syndrome (SARS) caused by a coronavirus (CoV), SARS-CoV, emerged into human populations in south China (Anon., 2003d; Peiris et al., 2003b,c; Poon et al., 2004a) from bats (Guan et al., 2003; Kan et al., 2005; Lau et al., 2005; Li et al., 2005d; Normile, 2005) in late 2002. Subsequently, SARS-CoV that had adapted to humans caused an epidemic in 29 countries and regions to which it had been carried by airline passengers. The epidemic was controlled by public health measures coordinated by the WHO and on July 5, 2003 it was officially declared to have ended. Because of these public health measures, a pandemic was averted (Enserink, 2003b). Close to 10% of the 8000 persons infected in this epidemic died. Molecular studies dissected the adaptation of this virus as it jumped from an intermediary animal, the civet, to humans, giving us valuable insights into processes of molecular emergence. Global research efforts are continuing to increase our understanding of the virus, the pathogenesis of the disease it causes (SARS), the "heterogeneity of individual infectiousness" (described below) as well as shedding light on how to prepare for other emerging viral diseases. Promising drugs and vaccines have been identified. The milestones achieved have resulted from a truly international effort.

The beginning of the epidemic and the identification of SARS-CoV

The epidemic began in Guangdong province, China, in late 2002. It spread to Hong Kong on February 21, 2003, and from there to other parts of the world. A week later, Carlo Urbani (Reilley et al., 2003), an Italian infectious disease expert working in the Hanoi, Vietnam, office of the WHO, responded to a possible avian influenza alert from French Hospital. That action by one man set into motion the engagement of the WHO, emergency measures by the Vietnamese government, and eventually the attention of the world. In Geneva, WHO team member Klaus Stöhr

(Stafford, 2005) put together and maintained a network of 11 microbiology laboratories in nine countries to respond to the epidemic and to identify the etiologic agent (Anon., 2003b).

Early encounters with SARS in Hong Kong suggested that a virus may have been the cause of the illness (Tsang et al., 2003a). Early candidate agents suggested were a paramyxovirus and a coronavirus, as well as the bacterial agent *Chlamydia pneumoniae* (Stadler et al., 2003). In the last week of March 2003, laboratories in Hong Kong (China), the United States, and Germany isolated a novel coronavirus from clinical material obtained from patients with SARS (Drosten et al., 2003a; Ksiazek et al., 2003; Peiris et al., 2003b). Serological studies and RT-PCR specific for this coronavirus (subsequently called SARS-CoV) were positive in most "probable" SARS patients but not in controls. RT-PCR products of several specimens from different geographical locations had identical nucleotide sequences, supporting the existence of a point-source outbreak. No other potential agent was consistently identified.

SARS-CoV could be grown in cell culture in Vero/African green monkey kidney cells (Drosten et al., 2003b; Ksiazek et al., 2003) and FRhK-4/fetal Rhesus kidney cells (Peiris et al., 2003b). The Hong Kong group led by Malik Peiris (Peiris, 2003) was the first to observe the cytopathic effect of the virus, seen after 2–4 days of incubation, consisting of cell rounding, refractile appearance, and detachment. The initial cytopathic effect was sometimes delayed until 6 days post-inoculation (Drosten et al., 2003a). (More recently, a clone of persistently infected Vero E6 cells has been established [Yamate et al., 2005].)

Work at Hong Kong University and the U.S. Centers for Disease Control and Prevention (CDC) resulted in the identification of the virus causing SARS. The CDC workers were the first to visualize the characteristic morphology of SARS-CoV in infected cells and in culture supernatant using transmission electron microscopy with negative staining (Fig. 1), which they shared with the network laboratories within 24 h (Anderson, 2005). With that information, the CDC successfully probed the virus with group I coronavirus polyclonal antibodies, and employed primers [IN-2(+), IN-4(−)] that targeted a conserved region of the coronavirus polymerase gene (open reading frame [ORF] 1b), thus amplifying the corresponding genomic region of SARS-CoV (Rota et al., 2003). Microarray hybridization further confirmed that the agent was a coronavirus. In Hong Kong, differential display priming (between SARS-CoV infected and uninfected cell cultures) and cloning were used to show that the virus was a coronavirus (Peiris et al., 2003b). German researchers performed random priming utilizing degenerate bases followed by sequencing and translated BLAST search to identify the RT-PCR products as those of a coronavirus (Drosten et al., 2003a).

Definitive proof of SARS-CoV as the etiologic agent of SARS came when Rotterdam virologists led by Albert Osterhaus (Enserink, 2003a) produced data that fulfilled Koch's last postulates. Macaque monkeys (*Macaca fascicularis*) developed a SARS-like illness after experimental infection, yielded the same virus inoculated, and developed a specific antibody response (Fouchier et al., 2003;

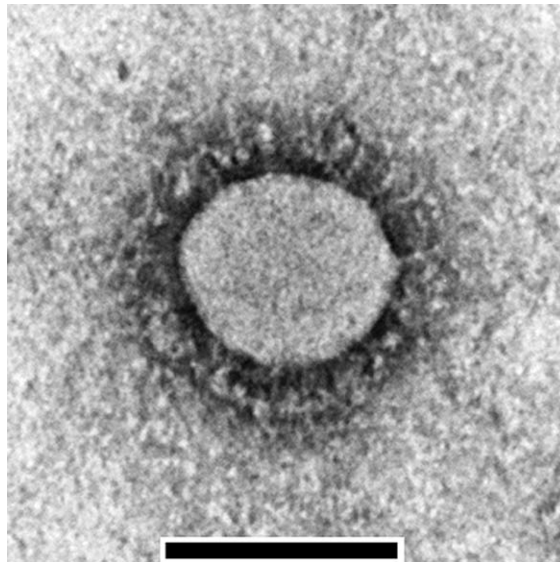

Fig. 1 Electron micrograph of SARS-CoV. The virus measures 60–120 nm in size. See text for description. Image generously provided by CDC/C.D. Humphrey and T.G. Ksiazek (US CDC Website-http://www.cdc.gov/ncidod/sars/lab/images.htm).

Kuiken et al., 2003). Co-infection of macaques with human metapneumovirus (hMPV), a virus that had earlier been a candidate agent for the cause of SARS, was not associated with more severe illness (Fouchier et al., 2003). hMPV infection without SARS-CoV caused only minor upper respiratory illness in adults (Ksiazek et al., 2003), although hMPV alone can cause severe pneumonia in young children (van den Hoogen et al., 2001). SARS-CoV was deemed necessary and sufficient to cause SARS.

On April 16, 2003, David Heymann (2004), Executive Director, WHO Communicable Diseases programs, Klaus Stöhr, and Albert Osterhaus announced that SARS was caused by the novel coronavirus, SARS-CoV (Anon., 2003b), and they dedicated the work to Dr. Urbani, who died from SARS that he had contracted while caring for patients in Vietnam.

The epidemic—timeline and highlights

SARS was notorious for a high incidence of acute respiratory distress and respiratory failure, a significant death rate even in healthy young adults (Lee et al., 2003b; Tsang et al., 2003a), a high rate of nosocomial transmission (Booth et al., 2003), and "superspreading events" (SSE) (Lai et al., 2004; Shen et al., 2004; Lloyd-Smith et al., 2005; Galvani and May, 2005) (Fig. 2). The epidemic in China almost became a pandemic when a physician guest at the Hotel Metropole, Hong Kong, who had been infected while treating SARS patients in Guangzhou, unknowingly

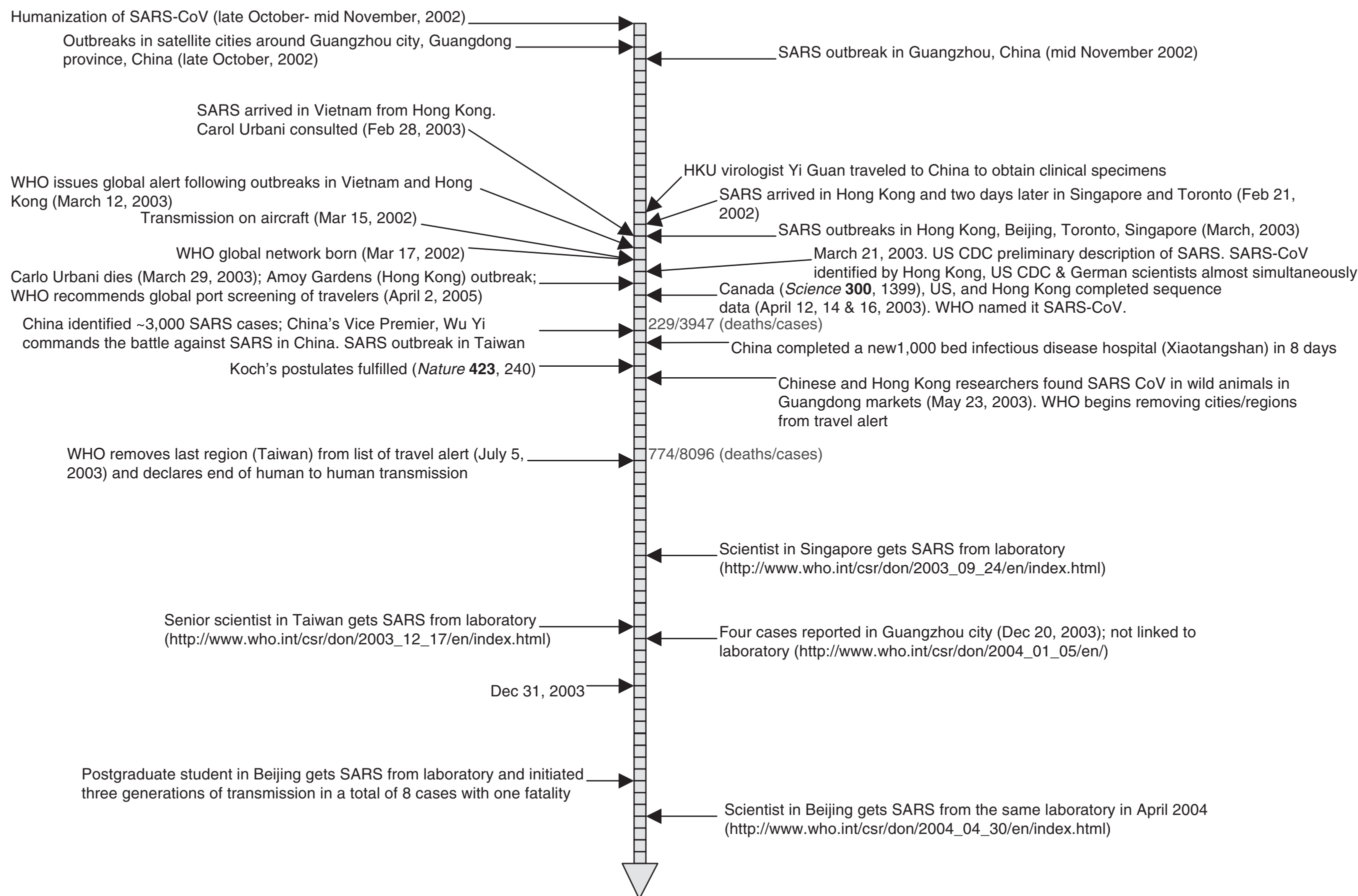

Fig. 2 Timeline of the SARS epidemic. Major events are listed from top to bottom. Each interval in the arrow represents 1 week. For information on the first weeks of the epidemic in China, consult the book by Thomas Abraham, Twenty-First Century Plague. The Story of SARS. The Johns Hopkins University Press. Baltimore, Maryland, 2005. (For colour version: see Colour Section on page 348).

introduced SARS-CoV into Hong Kong on February 21, 2003. He was probably a "superspreader" and Hong Kong became "ground zero."

However, in most other places where SARS-CoV spread, the chain of transmission stopped promptly with the isolation of patients. According to the Basic Reproductive Number (R_0), arrived at by averaging the number of infections produced by infected individuals in susceptible populations, SARS was not as contagious as influenza ($R_0 = 2.7$ [Riley et al., 2003] and 5–25, respectively). However, this simplification ignores a property of certain infectious diseases, including smallpox, influenza, and SARS, called the "heterogeneity of individual infectiousness." Highly variable infectiousness means that some infected individuals may cause explosive transmissions, giving rise to SSEs. During the SARS epidemic, a spectrum of infectiousness was seen that included SSEs, uneventful terminations of transmission chains, and explosive outbreaks (Galvani and May, 2005; Lloyd-Smith et al., 2005).

SSEs likely require high levels of viral shedding and others factors, which together determine the Individual Reproductive Number (Lloyd-Smith et al., 2005). One of these factors might be production of a large amount of bioaerosol by certain individuals (Edwards et al., 2004). Reducing bioaerosol by inhalation of nebulized saline (Edwards et al., 2004) and/or the use of cough suppressants could impact the Individual Reproductive Number and reduce the occurrence of SSEs.

Another cofactor might be a pneumonic phase with airborne dissemination of the virus (Lloyd-Smith et al., 2005). Because infectious disease agents exist as "quanta" and not as "plasma," airborne dissemination is difficult to prove owing to the stochastic process involved in the distribution of viruses by aerosol. During the Toronto portion of the SARS epidemic, investigations using state-of-the-art air sampling devices confirmed the presence of SARS-CoV in the air of a patient's room (Booth et al., 2005). These studies, together with data from investigation of transmission on aircraft (Olsen et al., 2003) and the huge outbreak of SARS in Amoy Gardens, Hong Kong (Yu et al., 2004b), showed that SARS-CoV is an opportunistic airborne pathogen (Roy and Milton, 2004). Having recognized that airborne dissemination of SARS-CoV is the route of transmission, facilities can be upgraded, with impact on other airborne infectious diseases as well.

The high incidence of nosocomial transmission of SARS-CoV during the epidemic exposed a weakness in the infection control procedures in some locations, as medical workers became vectors for SARS-CoV (Meng et al., 2005), but did not occur everywhere (Seto et al., 2003). Multiple layers of defense are needed, as Chowell et al. have suggested, because using their model for R_0, 25% of their R_0 distribution lies at $R_0 > 1$ even with perfect isolation (Chowell et al., 2004). Helpful measures might include the avoidance of crowding in clinics and wards, wearing face masks (Seto et al., 2003), avoiding aerosolizing procedures if possible (Tong et al., 2003; Tong, 2005b), improved ventilation design and rate (Liao et al., 2005a), and making sure that there are no "weak links" in infection control.

Emergence and origin of SARS-CoV

The theory that SARS-CoV came from an animal reservoir gained credence when field investigations by WHO showed that significant numbers of early patients were food-handlers (Anon., 2003c; Normile and Enserink, 2003; Xu et al., 2004c). Yi Guan and others investigated food markets in Guangdong, where a variety of small animals were kept in unhygienic heaped-up cages prior to sale (Guan et al., 2003). SARS-CoV-like coronaviruses were promptly identified in several Himalayan palm civets (*Paguma larvata*) and one raccoon dog (*Nyctereutes procyonoides*). Antibodies against SARS-CoV were also found in market workers. The relationship between these isolates from animals and isolates from humans appeared to be the result of a one-way transmission from animals to humans, because a 29-nucleotide deletion was found in the strain of SARS-CoV isolated from humans compared with civet SARS-CoV (it is easier to lose nucleotides than to gain some) (Chinese, 2004; Kan et al., 2005; Song et al., 2005b). Genomic comparisons further suggested that SARS-CoV was unlikely to be a recombinant between human and animal coronaviruses or between various animal coronaviruses, ruling out natural or laboratory chimerism (Holmes and Rambaut, 2004). Thus, SARS-CoV was probably a zoonotic virus (Holmes, 2003; Zhong et al., 2003b). It was also found that civets make a good amplification reservoir because SARS-CoV genomic RNA persisted in the spleen and lymph nodes of civets for as long as 35 days (Wu et al., 2005b).

Diversity of SARS-CoV genomes among human isolates was greatest in Guangdong, agreeing with animal studies that suggested south China was the site of emergence of the virus (Guan et al., 2004). Moreover, ‘‘humanization’’ likely occurred in a person of recent southern Chinese ancestry, because indigenous Taiwanese, with their distant HLA Class I genes, have been shown to be significantly less susceptible to SARS than residents of Taiwan who are immigrants from mainland China (Lin et al., 2003b). It is believed that the ‘‘humanization’’ of SARS-CoV occurred only a few weeks before the epidemic of SARS in China. The estimated dates of interspecies leap based on mutational analyses in both Singapore and China are in remarkably close agreement, late October 2002 and mid-November 2002, respectively (Chinese, 2004; Vega et al., 2004). The estimated mutation rates were 5.7×10^{-6} nucleotides per site per day in a Singapore isolate and 8.26×10^{-6} in a China isolate, again in remarkable agreement with each other and with the rate of 1.83×10^{-6} in a Taiwan isolate (Yeh et al., 2004). This rate of mutation is among the slowest in RNA viruses.

Retrospective seroepidemiological studies confirmed that SARS-CoV did not begin circulating in humans until recently. Only 1.8% of 938 sera collected in Hong Kong in May 2001 (Zheng et al., 2004b), none of 60 sera collected in Guangdong in early 2003 (Zhong et al., 2003b), and 1 (minimal reactivity on ELISA) of 384 sera from U.S. blood donors contained antibodies against SARS-CoV (Ksiazek et al., 2003; Zheng et al., 2004b). When quantified, titers of antibodies in these early sera were higher against civet SARS-CoV than against human isolates of SARS-CoV (Zheng et al., 2004b).

However, farmed civets elsewhere in China were mostly negative for SARS-CoV (Tu et al., 2004), so the hunt for the natural reservoir continued. Taking clues from other zoonotics, scientists turned to bats as a possible animal reservoir, since bats have been shown to be a reservoir for rabies virus, Ebola virus, Hendra virus, Menangle virus, and Nipah virus (Dobson, 2005; Leroy et al., 2005).

In 2005, two independent groups published definitive findings on the bat as a natural reservoir of SARS-CoV. Kwok-yung Yuen discovered three novel coronaviruses in different species of bat, including one virus with 88% nucleotide identity with SARS-CoV, a virus that they named bat-SARS-CoV (Lau et al., 2005; Poon et al., 2005). Bat-SARS-CoV, found in the insectivorous Chinese horseshoe bat (*Rhinolophus sinicus*), is nearly identical to civet SARS-CoV, including preservation of a 29-nucleotide segment not found in the majority of human isolates of SARS-CoV. Also nearly identical to civet SARS-CoV is SL-CoV Rp3, and perhaps related strains Rp1 and Rp2, found in *Rhinolophus pearsoni* by Li et al. (2005d). Shi, Zhang, and Wang's Sino-Australian cooperative effort, also involving Hong Kong University, produced proof that the bat is the natural reservoir for the SARS-CoV-like coronaviruses. These findings will lead to vaccines and drug treatments for SARS (Dobson, 2005).

Because SARS-CoV appears to jump species easily, more wildlife reservoirs of SARS-CoV may be discovered. Macaques, domestic cats, ferrets, raccoon dogs, pigs, and even mice are known to be susceptible to SARS-CoV infection (Fouchier et al., 2003; Martina et al., 2003; Wentworth et al., 2004; Chen et al., 2005b; Li et al., 2005d). Nevertheless, the fact that bats roost in large colonies makes them ideal reservoirs to maintain viruses and other microorganisms (Normile, 2005). In addition, bats are in the same Mammalia Class as humans, so viruses of bats will not require great changes to infect human cells (Li et al., 2005e).

After the epidemic was declared over, four small subsequent outbreaks occurred. Three were the result of SARS-CoV escaping from the laboratory by infecting personnel, as David Ho had predicted might occur (Enserink, 2003c), and has occurred with Russian influenza in 1977 (Horimoto and Kawaoka, 2005). The fourth case was a form fruste reemergence in the epicenter of the original outbreak, Guangzhou, between December 2003 and January 2004 (Enserink, 2004; Liang et al., 2004; Normile, 2004; Song et al., 2005b). In this reemergence, four people developed SARS and were confirmed to have SARS-CoV by RT-PCR. Three had had direct or indirect contact with palm civets, and one lived near a hospital that earlier admitted many patients with SARS. All recovered and seroconverted. Amplified sequences of the viruses isolated from them were very similar to those of SARS-CoV found in the preceding winter in caged animals (Chinese, 2004; Song et al., 2005b). The one patient in the reemergent outbreak who had had no contact with civets had earlier disposed of a dead rat, leading health officials of Guangdong to trap rodents near his residence; some of the rats (*Rattus rattus*) were found to have SARS-CoV in feces and lung tissue (http://www.egms.de/en/meetings/sars2004/04sars023.shtml), though not overtly ill.

The virus

Taxonomy and phylogeny

SARS-CoV belongs to the family *Coronaviridae*, which are enveloped RNA viruses in the order Nidovirales (Cavanagh, 1997). Coronaviruses are classified into three serogroups. Viruses in groups 1 and 2 are mammalian viruses; group 3 contains only avian viruses. Human coronaviruses (HCoV) are found in both group 1 (HCoV-229E and HCoV-NL63) and group 2 (HCoV-OC43 and CoV-HKU1) and are responsible for 30% or more of generally mild upper respiratory tract illnesses. To position SARS-CoV, Snijder et al. used a rooted phylogenetic tree that included an outgroup, the equine torovirus (EToV) (Snijder et al., 2003). They concluded that SARS-CoV is distantly related to established group 2 coronaviruses, agreeing with Peiris's phylogenetic analysis using the polymerase gene (Peiris et al., 2003b). Most of the genome of SARS-CoV is closely related to group 2 coronaviruses (Magiorkinis et al., 2004). Now SARS-CoV is placed in a new subgroup 2b, with the other group 2 coronaviruses assigned to a new subgroup 2a (Stadler et al., 2003; Gorbalenya et al., 2004). In addition, bat-SARS-CoV was assigned recently to subgroup 2b (Lau et al., 2005).

Ultrastructure of SARS-CoV

SARS-CoV has the characteristic morphology of coronaviruses, with spike (S) protein peplomers, club-shaped projections on the surface, giving the enveloped viral particle a crown-like (hence "corona," Latin for "crown") appearance under the electron microscope (Fig. 1). Atomic force microscopy reveals that each virion has at least 15 spherical spikes, each with a diameter of $7.29 +/- 0.73$ nm (Lin et al., 2005a). The center appears amorphous.

SARS-CoV genome, proteome, and replication cycle

Coronaviruses have the largest known non-segmented genome among RNA viruses (27–31 kb). The genome mimics eukaryotic mRNA in being single-stranded positive-sense RNA, capped and methylated at the 5′ end, and polyadenylated at the 3′ end. Consequently, it is more stable than prokaryotic mRNA, and is optimized for translation by eukaryotic translational machinery (i.e. optimized for infectivity). A polymerase is not included in the particle.

The entire genome sequence of SARS-CoV was worked out in <2 months (Leung, 2003; Marra et al., 2003; Rota et al., 2003). The SARS-CoV genome (Fig. 3) begins and ends with untranslated regions (UTR), spanning 192 and 340 nucleotides, respectively. (In the Sabin strain of poliovirus, mutations in the UTR were responsible for the attenuation that permitted vaccine production (Gutierrez et al., 1997). The SARS-CoV genome has 14 predicted open reading frames (ORFs) encoding 28 proteins (Marra et al., 2003; Rota et al., 2003; Snijder et al., 2003).

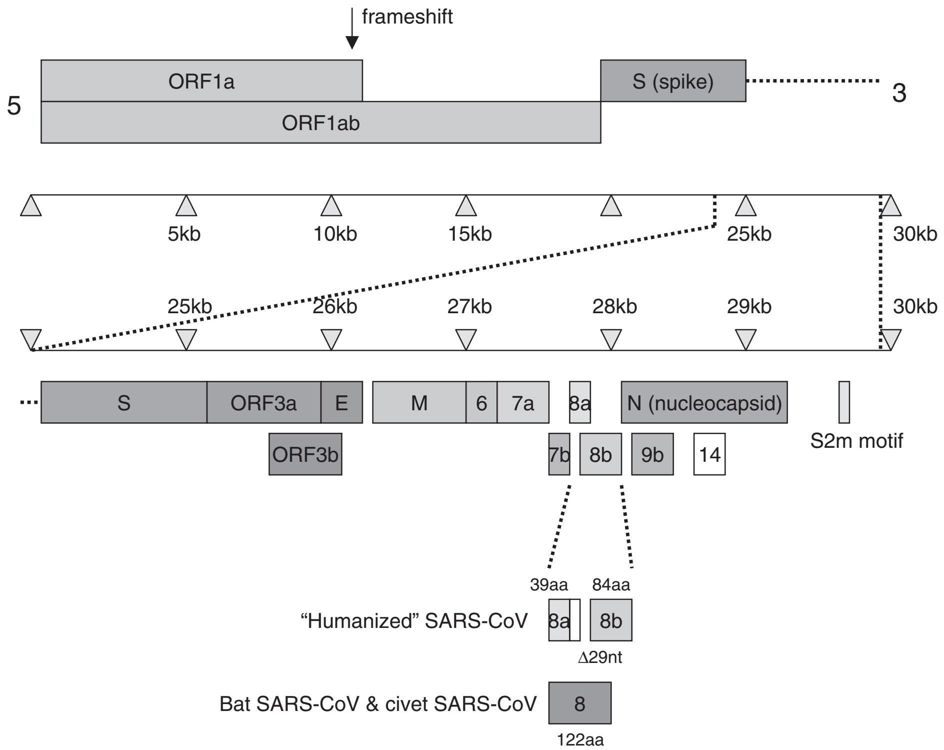

Fig. 3 SARS-CoV genome organization. The genome organization is similar to other coronaviruses with respect to overall size, the relative positions of replicase, spike, envelope, membrane and nucleocapsid genes, and certain other features (see text for details). A 29-nucleotide stretch is deleted in the strain found in human isolates, as illustrated at the bottom. (For colour version: see Colour Section on page 349).

Certain strains lack one ORF (Chinese, 2004), whereas others have 15 ORFs (Groneberg et al., 2005). There are alternative nomenclatures related to this virus, which in this chapter are enclosed within brackets.

A "SARS chip" was offered free to researchers beginning June 23, 2003, by the National Institute of Allergy and Infectious Diseases, NIH (USA), based on the success of the DeRisi "viral discovery microarray" (Wang et al., 2003). Microarray gene expression studies on peripheral blood have been shown to discriminate accurately SARS patients from non-SARS controls (Long et al., 2004; Lee et al., 2005b).

In Shanghai, the proteome of SARS-CoV in Vero cells has been analyzed using conventional proteomic tools and two-dimensional liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). In addition, isotope-labeled affinity tag technology coupled with two-dimensional LC-MS/MS has

been used to identify and quantify 186 differentially expressed proteins in infected vs. non-infected Vero cells (Zeng et al., 2004b; Jiang et al., 2005b). In Beijing, Kang et al. developed a mass spectrometry decision tree classification algorithm using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) and protein array, predicting the virologic diagnosis based on several serum proteomic markers (Kang et al., 2005). Using similar technology, Poon et al. at Chinese University of Hong Kong (CUHK) performed serial analyses of plasma proteomic signatures in pediatric patients with SARS, showing the potential to predict clinical outcome based on its correlation with viral load (Poon et al., 2004c).

Replicase

ORF1a and ORF1b, together spanning two-thirds of the viral genome, are located at the 5′ end and encode replicase polyproteins (pp) 1a and pp1ab. ORF1 is expressed immediately after infection. Translational products can be detected in cell cultures as early as 6 h after infection (Prentice et al., 2004). Like other coronaviruses and many other viruses, the strategy used for translation of pp1ab requires a "slippery sequence" and a structural mRNA element known as a pseudoknot, which causes a 1-ribosomal frameshift just 5′ of the termination codon of ORF1a. The "slippery sequence" is highly conserved and would not function if mutated (Thiel et al., 2003). Another *in cis* element has been discovered, an attenuator 5′ of the slippery sequence that downregulates 1 frameshift efficiency (Su et al., 2005). Transcription attenuation was thought to enable SARS-CoV to synthesize both full-length and subgenomic-length antisense RNA intermediates (Yount et al., 2005).

Sixteen non-structural proteins (nsp) are derived from proteolytic cleavage of pp1a and pp1ab by two, rather than three, viral proteinases (see below) (Snijder et al., 2003). Recently, 12 of the 16 predicted nsp have been identified by immunoblot and their subcellular localization studied by immunofluorescence confocal microscopy (Prentice et al., 2004).

The predicted 20-kD nsp1 has been confirmed, along with immunologically related products of different sizes, and are awaiting further characterization (Prentice et al., 2004). Nsp2 is not essential for viral replication in Vero cells (Graham et al., 2005), which are defective in interferon (IFN) production (Emeny and Morgan, 1979). The two viral proteases, a very specific papain-like cysteine proteinase (nsp3, PL2pro; SARS-CoV does not have PL1pro), and the main chymotrypsin-like protease (nsp5), also known as 3C-like cysteine proteinase (3CLpro), with substrate specificity conserved among coronaviruses, are necessary for co- and post-translational processing of the polyprotein. PL2pro cleaves nsp1, nsp2, and nsp3 from the elongating polypeptide co-translationally, whereas 3CLpro is responsible for the other cleavage sites. The X-domain of nsp3 is homologous to adenosine diphosphate-ribose 1′-phosphatase (ADRP), which is involved in pre-tRNA splicing (Snijder et al., 2003). Its phosphatase activity was recently

demonstrated in *in vitro* assays (Saikatendu et al., 2005). Nsp4 is a hydrophobic protein of 35 kDa on SDS-polyacrylamide gel but has a predicted mass of 55 kDa. It is similar to MP1 of murine hepatitis virus (MHV) (Prentice et al., 2004). The use of ''artificial neural networks'' and SPAAN, a bioinformatics software that has a track record of discovering adhesins, has led to the finding of adhesin-like characteristics in nsp6, a function also ascribed to spike, nsp2, nsp5, and nsp7 (Sachdeva et al., 2005). Nsp7 and nsp8 assemble into a cylindrical hexadecamer with inner positive charges and an internal diameter of 30 Å, thought to confer processivity to RNA-dependent RNA polymerase (RdRp) (Zhai et al., 2005). Other scientists have cloned nsp9, expressed it in *E. coli*, crystallized it, and generated crystallographic data in an effort to facilitate drug design (Campanacci et al., 2003). They further showed that it is a single-stranded RNA-binding protein displaying an oligosaccharide/oligonucleotide fold unique to the world of RNA viruses (Egloff et al., 2004). Nsp12 functions as SARS-CoV RNA-dependent RNA polymerase (RdRp or POL). Nsp13 is a promiscuous helicase (unwinds RNA and DNA) with ATPase activity belonging to the helicase superfamily 1 (Thiel et al., 2003).

The N-terminal domain of nsp14 may play a role in the stability of the SARS-CoV genome. The N-terminal domain of nsp 14 is homologous to 3′–5′ exonulcease (ExoN) and it has been speculated that it may perform functions such as RNA proofreading, repair, and/or recombination (Snijder et al., 2003). That SARS-CoV has this genome protection capability may explain the slow estimated mutation rate (Chinese, 2004; Vega et al., 2004; Yeh et al., 2004), the sequence identity between a laboratory-acquired case and a stable laboratory isolate (Vega et al., 2004), and the observed but unexpected resistance to ribavirin, a drug that predisposes other RNA viruses to go into ''error catastrophe'' (Eigen, 1987; Crotty et al., 2001; Pariente et al., 2001). Nevertheless, preliminary observations suggest that quasispecies do occur with SARS-CoV just as with other RNA viruses, although all patients studied for quasispecies existence had all received ribavirin (Xu et al., 2004a,b), a confounding factor in the experiments.

The C-terminal part of nsp15 is homologous to poly(U)-specific endoribonuclease (XendoU), involved in small nucleolar RNA processing and utilization (Snijder et al., 2003). Nsp15 was further confirmed to have specificity for cleavage at uridylate residues. Structural analysis shows that it is arranged as a dimer or trimer with possible RNA-binding sites (Guarino et al., 2005). Nsp16 shows homology with 2′-*O*-methyltransferase (2′-*O*-MT) (von Grotthuss et al., 2003), and could possibly be used to cap the viral mRNA, disguising it as a eukaryotic mRNA. The cap is recognized by eukaryotic initiation factor eIF4F, which together with other initiation factors, recruit the 43S ribosomal subunit initiation complex that then scans the mRNA for the initiation codon AUG, whereupon the 60S ribosomal subunit docks and begins peptide elongation (Wang et al., 1997). Co-localization studies showed that these enzymes assemble into one or more vesicle membrane-associated replication units (Prentice et al., 2004) and usurp cellular processes for viral replication (without a DNA phase) and mRNA transcription.

Expression of other SARS-CoV genes

Coronaviruses, including SARS-CoV and other viruses in the order Nidovirales, produce a set of 3′ co-terminal mRNA in host cells (Lai and Cavanagh, 1997). For SARS-CoV, the 12 ORFs downstream of the replicase are translated from a nested set of eight subgenomic mRNAs, identified experimentally in infected cells (Snijder et al., 2003; Thiel et al., 2003). Also like other coronaviruses, all of the subgenomic mRNAs can be lined-up with the genome at (are co-terminal with) the 3′ end of the virus. The 5′ ends are also identical. A minimal consensus sequence at the 5′ end, 5′ACGAAC-3′, is sufficient to direct the synthesis of the subgenomic mRNAs (Thiel et al., 2003). This sequence, embedded in a stretch of nucleotides upstream of the ORF(s) of each subgenomic mRNA, is the result of fusion of nucleotides 1–72 of the genome (“leader” transcription regulatory sequence [TRS]) and a unique “body” TRS preceding each ORF. The joining of these two regions is probably achieved in a discontinuous step during minus-strand synthesis (Stadler et al., 2003; Thiel et al., 2003). The TRSs regulate viral transcription and translation.

Spike protein and receptor-based entry

ORF2 encodes a Class I viral membrane-fusion protein, the 1255-amino acid transmembrane S protein. The precursor, proS, is glycosylated at the Golgi apparatus and proteolytically processed by furin (host membrane-bound proprotein convertases), as shown by scientists at the Clinical Research Institute of Montreal (Bergeron et al., 2005). The extracellular domain contains the S1 receptor-binding and the S2 entry-mediating “regions.” Breaching the cell membrane barrier is the single most important step in infection (Giroglou et al., 2004; Li et al., 2004a). Neutralizing antibodies against S, especially the receptor-binding region, prevents infection (He et al., 2004b,c).

S binds the host cell membrane protein angiotensin I converting enzyme 2 (ACE2) (Dimitrov, 2003; Li et al., 2003; Kuba et al., 2005), which the newly discovered HCoV-NL63 also employs as its receptor (Hofmann et al., 2005). Civet ACE2 (cACE2) differs from human ACE2 (hACE2), to which human isolates of SARS-CoV are not fully adapted, as shown by increased binding and infection by introduction of residues 90–93 of cACE2 into the human receptor (Li et al., 2005e). As expected, soluble ACE2 (sACE2) but not soluble ACE (sACE), blocks binding of S with Vero E6 cells (Li et al., 2003; Hofmann et al., 2004). However, mutations of the catalytic site of ACE2 have no effect on S-induced syncytia formation, suggesting that existing ACE inhibitors will not block SARS-CoV infection (Dimitrov, 2003).

The receptor-binding region of S has been localized to amino acid residues between 318 and 510 of the S1 “region” (Wong et al., 2004b), in the same neighborhood as that of HCoV-229E (Breslin et al., 2003). The avidity of binding (Li et al., 2005e), viral entry (Yi et al., 2005), and immunogenicity (Yi et al., 2005) are affected by single amino acid substitutions within this region, as demonstrated

at 2.9 Å resolution (Holmes, 2005; Li et al., 2005b). Binding is by way of conformational and electrostatic interactions (Yi et al., 2005). The strength differs, depending on the viral strain. Civet S binds cACE2 avidly but hACE2 poorly, although S from human isolates of SARS-CoV binds strongly with both cACE2 and hACE2 (Li et al., 2005e). Two regions of the S-protein-binding site on ACE2, and two residues (aa479 and aa487) in the receptor-binding region of S, largely determine the difference. S from human isolates of SARS-CoV has a small neutral residue (asparagine) at position 479, replacing a basic residue in civet S, and better accommodates the lysine at position 31 of hACE2. At position 487, serine in civet S is replaced by threonine, which has an extra methyl group, in the human isolates. This residue was absolutely conserved in S evaluated in >100 isolates during the epidemic (Li et al., 2005e). These findings suggest that adaptation of S to hACE2 was critical to viral adaptation to humans (Ruan et al., 2003).

After binding to ACE2, S undergoes conformational change mediated by its S2 "region" (Liu et al., 2004; Tripet et al., 2004; Xu et al., 2004d), which presumably exposes a cleavage site, followed by pH-dependent host endosomal cathepsin L-mediated proteolysis (Yang et al., 2004a; Simmons et al., 2005). Extracellular proteases, such as those excreted by neutrophils, facilitate this pathway to a significant degree. Proteases also enable entry into the cell of SARS-CoV adsorbed on the cell surface (Matsuyama et al., 2005). In a similar way, Ebola virus and Hendra virus also utilize cathepsin L for the endocytic pathway of cell entry (Chandran et al., 2005; Pager and Dutch, 2005).

The final step of membrane fusion before viral entry is dependent on the formation of the six-helix bundle by heptad repeats (HR1 and HR2) in the S2 "region" (Liu et al., 2004), also predicted by others using in-silico techniques (Kliger and Levanon, 2003). Oligomerization of S is required to form the six-helix bundle, a structure similar to the fusogenic core of HIV-1 gp41 (Liu et al., 2004). The putative fusion peptide has been located to the region immediately upstream of HR1 (Bosch et al., 2004) and provided detailed modeling of the fusion core (Supekar et al., 2004; Xu et al., 2004e; Guillen et al., 2005). This last step of viral entry appears to be susceptible to inhibition by a component of innate immunity, mannose-binding lectin (MBL) (Leikina et al., 2005).

Several other cellular proteins are also utilized by S to facilitate entry into the cell. They include the HIV-attachment factor dendritic cell-specific intercellular adhesion molecule-grabbing non-integrin (DC-SIGN or CD209), a C-type lectin expressed on dendritic cells, and CD209L (L-SIGN or DC-SIGNR) (Jeffers et al., 2004; Marzi et al., 2004). In this way SARS-CoV joins HIV, dengue virus, and CMV in exploiting dendritic cells as "vehicles" for cell-mediated dissemination (Yang et al., 2004a). Another lectin (LSECtin) was recently shown to bind to S and could assist viral entry into the liver and lymph nodes through its expression in sinusoidal endothelial cells (Gramberg et al., 2005).

In a short period of time, SARS-CoV S has evolved from one that is inefficient in entering human cells (civet strains) to one that has acquired "keys to both the front and back doors." This is no less remarkable than influenza virus strains that

have acquired the ability to efficiently enter human cells through mutations of the hemagglutinin cleavage site that became promiscuously cleaved by a wide range of host proteases (Horimoto and Kawaoka, 2005).

Other structural proteins

ORF4 [5] encodes a small envelope (E) protein of 76 amino acids (Shen et al., 2003) that binds to and inactivates the anti-apoptotic protein, Bcl-xL, causing *in vitro* and *in vivo* lymphocytotoxicity (Yang et al., 2005b). E protein was detected in culture supernatant (Hsieh et al., 2005) and could conceivably be taken up *in vivo* by lymphocytes that are not infected by the virus.

ORF5 [6] encodes membrane (M) protein, the most abundant viral glycoprotein (221 amino acids). It is a 3-span transmembrane protein with a short N-terminal ecto- and a long C-terminal interior domain. A 12-amino acid domain of M has been shown to interact with the nucleocapsid, triggering viral encapsidation (He et al., 2004a; Fang et al., 2005).

ORF9a [12] encodes the nucleocapsid (N) protein (422 amino acides). It is a basic protein that binds to viral RNA via its N- and C-terminal regions (Chang et al., 2005; Hsieh et al., 2005). It spontaneously assembles into virus-like particles (VLP) in solution (Azizi et al., 2005). A role in apoptosis has been postulated (Surjit et al., 2005).

Accessory proteins

The functions of the accessory proteins are still unknown (Cai et al., 2005). Systematic deletions of 5 of 8 group-specific ORFs, ORF3a, OF3b, ORF6, ORF7a, and ORF7b, alone or in combinations, did not significantly impair viral viability in cell culture and in mice (Yount et al., 2005). It is possible that they are involved in the struggle between the virus and its natural host, the bat. Deletion of group-specific ORFs from other coronaviruses often leads to attenuation *in vivo* (Yount et al., 2005). Evidence is emerging that some of these deletions might be pathogenic in man. ORF3a [3] and ORF3b [4] encode U274 (sars3a) and U154 (sars3b), respectively, two novel proteins not found in other coronaviruses (Tan et al., 2004b; Yu et al., 2004a; Ito et al., 2005). Both are implicated in triggering apoptosis (Law et al., 2005b; Yuan et al., 2005). U274 (sars3a) co-localizes with S and M and is packaged into virions (Ito et al., 2005). U122 (sars7a) encoded by ORF7a [8] (Snijder et al., 2003) is also localized to the rough endoplasmic reticulum/Golgi compartment. It has been reported to induce apoptosis by a caspase-dependent mechanism (Tan et al., 2004a). It is tempting to associate these apoptosis-inducing activities with lymphopenia, in a "Trojan horse" role. Infection of lymphocytes by SARS-CoV might be needed for survival (Gu et al., 2005); by causing lymphocyte apoptosis, virus with these deletions might permit survival of the virus in other cells that would otherwise be destroyed by the lymphocytes.

Viral assembly and exit

Assembly of viral particles is triggered by the association of membrane-bound M proteins with N proteins that coat the helical viral nucleocapsid (He et al., 2004a). Viral packaging is dependent on viral RNA-binding motifs at the N- and C-terminals of N and a packaging signal in the hypervariable region of ORF1b near the 3′ terminus, similar to that found in MHV and BCoV (Qin et al., 2003; Chang et al., 2005; Hsieh et al., 2005). The virions assemble and bud into vesicles formed in the compartment between the endoplasmic reticulum and the Golgi apparatus, picking up carbohydrate moieties before being released by exocytosis. The entire process of SARS-CoV replication takes from 9 to 24 h (Yount et al., 2003; Prentice et al., 2004), compared with the 5–6 h for influenza virus, as reflected by the slow rise in viral titers in SARS patients (Peiris et al., 2003a; Tsang et al., 2003b; Cheng et al., 2004).

Viral countermeasures

Microarray studies of host gene expression have shown that SARS-CoV elicits a non-specific innate inflammatory reaction rather than an antiviral response (Reghunathan et al., 2005). SARS-CoV in a sense "edits" the responses of dendritic cells and macrophages, resulting in induction of non-specific inflammatory chemokines, a low-level pro-inflammatory cytokine production, and a muted anti-viral cytokine response (including IFN and IL-12p40). Data have suggested that in fact SARS-CoV avoids activating dendritic cells and macrophages (Ziegler et al., 2005). A suppressed Th-2 response, which would otherwise orchestrate the humoral immune response, could also result (Wong et al., 2004a).

In search of an explanation for the absent IFN-β response, it has been found that SARS-CoV blocks a step after the early nuclear transport of IRF-3, a key IFN-β gene transcription factor (Spiegel et al., 2005). Another cellular signaling pathway affected is the activation of AP-1 by S and N (He et al., 2003; Chang et al., 2004). N protein activates nuclear factor (NF)-kB in Vero E6 cells but not Vero cells, HeLa cells, or lung epithelial and fibroblast cell lines (Chang et al., 2004; Liao et al., 2005b). Other viruses that activate NF-kB include HIV-1, human T-cell lymphotropic virus type 1, herpes viruses, hepatitis C virus, hepatitis B virus, and influenza viruses, among others (Santoro et al., 2003). The protean roles of NF-kB in inflammation, cell proliferation, cell survival, apoptosis, and as a central regulator of innate and adaptive immune responses, make it an ideal intracellular target for the virus.

The most important aspect of SARS-CoV evasion of the immune response occurs by abrogation of intracellular antiviral responses, aptly described as "intracellular warfare" (Krug et al., 2003), which also occurs in most viral infections (Samuel, 2001; Webby et al., 2004). Vero cells stably expressing MxA (Samuel, 2001), a broad-spectrum anti-RNA virus large GTPase protein induced by IFNs α and β (but not γ), continue to produce the virus, suggesting that SARS-CoV has

countermeasures against this important defense mechanism (Spiegel et al., 2004). The adaptive immune system is itself a viral target; direct viral infection of CD4+ and CD8+ lymphocytes (Gu et al., 2005), induction of lymphocyte apoptosis (Chen et al., 2005a), and the multiple toxic viral molecules that can cause apoptosis, namely E protein (Yang et al., 2005b), U274 (Law et al., 2005b), U154 (Yuan et al., 2005), U122 (Tan et al., 2004a), and S (Chow et al., 2005). Harnessing the power of computational biology, scientists at the Beijing Genomics Institute are investigating viral virulence and evasion strategies by comparing SARS-CoV protein motifs with those of known human proteins and other viral proteins. Toxic motifs were found to be localized to various SARS-CoV proteins potentially relevant for therapy and vaccine design (Li et al., 2004b). For example, superantigen-like motifs in the S, E, N, and some accessory proteins are thought to cause lymphocyte apoptosis by activation in the absence of appropriate co-stimulatory signals. An enterotoxin motif in S could account for the diarrhea seen in many SARS victims.

More viral countermeasures may be identified in the future, perhaps including anti-RNA interference (RNAi). Progress is being made in the application of RNAi-based therapeutics against SARS-CoV (Zhang et al., 2003; Wang et al., 2004c; Li et al., 2005a; Shi et al., 2005; Tao et al., 2005; Wu et al., 2005a), in particular the recent advances in delivery of siRNAs, with the hope of specifically targeting the scattered and relatively inaccessible lymphocytes and dendritic cells to ameliorate the assault on the immune system by SARS-CoV (Song et al., 2005a).

Molecular evolution

Comparisons of the genomes of SARS-CoV and SARS-CoV-like animal coronaviruses from palm civets and a raccoon dog (Guan et al., 2003) revealed that human isolates of SARS-CoV all shared some deletions not seen in civet SARS-CoV. Additional polymorphisms were also identified, showing that humanized SARS-CoV had evolved from civet SARS-CoV (Chen et al., 2005b). These initial efforts also cataloged important genomic data for comparison with newly discovered SARS-CoV-like viruses, such as bat-SARS-CoV (Lau et al., 2005) and SL-CoV Rp3 (Li et al., 2005c). Both of these bat-SARS-CoVs contained the sequences deleted in humanized SARS-CoV, revealing their even closer kinship with civet SARS-CoV.

The Chinese SARS Molecular Epidemiology Consortium collected spatially and temporally diverse specimens from humans and animals to study SARS-CoV evolution (Chinese, 2004). Several major SARS-CoV genotypes and 299 single-nucleotide variations (SNVs) were discovered among 63 sequences studied. Certain SNVs, for example, the second and third nucleotides of codon 479 of S (22927 and 22928) created the so-called multiple substitution codons, which have a chance occurrence of zero, pointing toward their development by natural selection.

Another observation was the phenomenon of G + C enrichment (Song et al., 2005b), thought to be metabolically expensive for intracellular pathogens (Rocha and Danchin, 2002), but which also could be due to pressure from host sabotage akin to G → T editing by host APOBEC3 family proteins, again suggesting adaptive pressure rather than random mutations. Genotyping based on SNVs in 5 loci (17564, 21721, 22222, 23823, and 27827) defined civet SARS-CoV (GACGC), an early GACTC motif in human isolates of SARS-CoV, GGCTC, or GATTC motifs in the middle of the epidemic, and a late TGTTT motif that has corresponding non-synonymous changes in ORF1b, S and the non-coding X3 (ORF6 or ORF7) regions (Liu, 2005). Similar changes occurred in the evolution of the three most significantly variable proteins S, sars3a (U274), and nsp3 (PL2pro), as the virus adapted to humans (Kan et al., 2005; Song et al., 2005b). Sequence deletions were observed in the dominant human strain, including entire ORFs specifying group-specific accessory proteins, such as a 29-nucleotide deletion in the ORF8 of civet SARS-CoV, creating ORF8a and ORF8b. An uncommon strain with limited range isolated early in the epidemic had an 82-nucleotide deletion in ORF8a, similar to animal SARS-CoV isolated in farmed civets in Hubei province, China (Chinese, 2004). Yet another strain isolated late in the epidemic in Hong Kong in May 2003 lacked a 415-nucleotide sequence corresponding to the entire ORF8a and ORF8b (Poon et al., 2004a).

These and other data indicate that there was a rapid viral genomic adjustment to the human host early in the epidemic, particularly at specific mutational hotspots in the receptor-binding region. This slowed in the middle of the epidemic, with late changes producing strong purifying selection (Chinese, 2004; Yeh et al., 2004; Kan et al., 2005). These investigations also revealed that critical viral adaptations to humans went down the same evolutionary path in Beijing as earlier in Guangzhou, with a switch from the GGCTC to the TGTTT motif in patients belonging to the same cluster (Liu, 2005). Some of these molecular events occurred again in the late 2003 to early 2004 outbreak in Guangzhou (Kan et al., 2005; Song et al., 2005b). Throughout this period, civet SARS-CoV had been evolving and adapting to civet cats, producing a higher viral load, confirming that the civet is not its normal reservoir (Song et al., 2005b). However, given the opportunity, it appeared that SARS-CoV could establish a reservoir in farmed civets. These findings and the elucidation of the adaptation of S to hACE2 sharpen our perception of molecular emergence.

Because the human isolates of SARS-CoV are much more virulent and contagious than the animal strains, it is important for public health officials to quickly find out which strain(s) they are dealing with in the event of an outbreak. As such, the genotyping of SARS-CoV has acquired public health significance. Scientists at the Chinese University of Hong Kong have developed an allelic discrimination genotyping assay using 5′ nuclease probes that has been validated for discriminating between human and animal strains of SARS-CoV, by comparison with direct sequencing (Chung et al., 2005).

Human disease

Transmission

It is thought that SARS-CoV spreads from person to person through mucosal surfaces with virus-laden body fluids, primarily respiratory secretions (Anon., 2003a). Tears (Loon et al., 2004; Tong and Lai, 2005) and sweat (Ding et al., 2004) have also been found to contain SARS-CoV RNA, although their role in disease transmission is unknown. Coughing as a means of airborne dissemination was difficult to prove (Seto et al., 2003; Tong, 2003), but was demonstrated experimentally (Booth et al., 2005). Large droplets emitted by coughing and sneezing and having a projection range of 1 m are believed to have contributed to airborne transmission (Fowler et al., 2004; Yu et al., 2004b, 2005; Booth et al., 2005; Li et al., 2005f,g; Tong, 2005a), possibly by superspreaders (Edwards et al., 2004); inadvertent aerosolization of other infected body materials or feces is also thought to be a possible means of transmission (Yu and Sung, 2004; Yu et al., 2004b). Fecal-to-oral transmission has not been documented, although viral nucleic acid has been found in sewage (Wang et al., 2005a). Nuclei acid testing by RT-PCR, if instituted, theoretically could prevent transfusion-associated transmission during future epidemics if SARS-Cov is shown to be present in blood from asymptomatic persons (Schmidt et al., 2004).

Clinical features of SARS

The clinical course has been divided into three phases (Peiris et al., 2003a). Phase 1 correlates with upper respiratory viral replication and viremia. Phases 2 and 3 correlate with lower respiratory tract viral replication, with phase 3 characterized by functionally critical pulmonary injury, due either to virus alone (Mazzulli et al., 2004) or in conjunction with immunological damage (Lin et al., 2005b; Matsuyama et al., 2005).

The incubation period of SARS ranges from 2 to 11 days after exposure, with a mean of 4.6 days (Leung et al., 2004), and occasionally was as long as 16 days (Booth et al., 2003; Lee et al., 2003b; Tsang et al., 2003a). The prodrome consists of flu-like symptoms such as malaise, myalgia, headache, fever, and rigors. Cough as an initial symptom is present in greater than half of the patients. Coryza and sore throat are present in a minority of cases. By day 5 (range, 3–7 days), up to 76% of patients develop exertional dyspnea; chest X-rays reveal opacities in the lungs of almost all patients, consistent with alveolar and interstitial exudation (Zhong et al., 2003b). This phase lasts for up to a week. Watery diarrhea developed in 20.4% of patients admitted to Princess Margaret Hospital, Hong Kong (Kwan et al., 2005a). A higher incidence of diarrhea was found in the cohort of patients from the Amoy Gardens (74%) and correlated with higher viral load in stool (Hung et al., 2004).

More than 20% of patients require intensive care, and 13% require ventilatory support (Tsui et al., 2003).

Asymptomatic cases were extremely rare during the epidemic (Lai et al., 2005b), being the rule in the early phase of the epidemic in China, before SARS-CoV had adapted to the human host (Guan et al., 2003; Lee et al., 2003a). Some elderly patients were not febrile (Christian et al., 2004); in some cases, the incubation period and convalescence was protracted. Up to 5.8% of 138 patients in Prince of Wales Hospital presented with diarrhea and fever without respiratory symptoms (Leung et al., 2003b).

Children were also infected, although their symptoms and clinical course were typically mild (Hon et al., 2003; Leung et al., 2003a; Kwan et al., 2004). Pregnancy did not alter the course of SARS in the mother and perinatal infection has not been reported (Shek et al., 2003). Children have also presented solely with diarrhea (Kwan et al., 2005b).

Recovery, if it will occur, begins between 14 and 18 days after disease onset (Christian et al., 2004). Convalescent patients no longer excrete the virus in stools (Wang et al., 2005b). In some patients, apparent recovery is followed by exacerbation (Peiris et al., 2003a), with arterial oxygen desaturation, and variable degrees of respiratory impairment or failure. At one year post-infection, one-third of patients continue to have pulmonary function impairment (Hui et al., 2005; Ong et al., 2005). Osteonecrosis occurs in a significant proportion of patients who receive systemic steroids; children can also experience this complication (Chan et al., 2004a; Griffith et al., 2005).

Clinical laboratory findings

Lymphopenia (Peiris et al., 2003b; He et al., 2005b) and thrombocytopenia are commonly present (Choi et al., 2003). Decrease of both CD4+ and CD8+ T-lymphocytes occurs early and adversely affects the prognosis (Wong et al., 2003a). Serum chemistry often reveals elevated lactate dehydrogenase (LDH), aminotransferase levels, and creatine phosphokinase levels, with LDH being an independent predictor of mortality (Choi et al., 2003). Initially elevated C-reactive protein levels were reported to be correlated with poor outcome (Wang et al., 2004a).

Pathology of SARS-CoV pneumonia

In humans, ACE2 is expressed in the lungs, intestines, testes, kidneys, endothelium, and heart, and in large part determined the tissue tropism (Donoghue et al., 2000; Tipnis et al., 2000; Harmer et al., 2002; Leung et al., 2003b; Hamming et al., 2004; Xu et al., 2005). In respiratory epithelium, ACE2 is expressed only in differentiated cells (Jia et al., 2005), in particular, ciliated cells (Sims et al., 2005), implying that bronchioalveolar stem cells (Kim et al., 2005) are poorly infected.

Abundant evidence points to viral infection of pneumocytes as the mechanism of lung injury (Nicholls et al., 2003; To et al., 2004; Chan et al., 2005b; Shieh et al., 2005). However, induction of an immune response by viral proteins is also

implicated in the pathogenesis. For example, the N-terminal aa324–488 and the C-terminal aa609–688 of S activate AP-1 and MAPKs independent of NF-kB in lung epithelial cells, monocytes, and fibroblasts *in vitro* leading to induction of IL-8 (Chang et al., 2004), consistent with observations that IL-8 and IL-2 levels are elevated in patients with SARS (Lee et al., 2004). In turn, IL-8 recruits neutrophils to the lungs, thereby facilitating SARS-CoV entry into pneumocytes by elaboration of proteases (Chang et al., 2004; Wong et al., 2004a; Matsuyama et al., 2005; Tang et al., 2005).

Host factors appear also to contribute to diffuse alveolar damage. The peptide hormone angiotensin II, positively and negatively regulated by ACE (product of ACE1 gene) and ACE2, respectively, has been shown to participate in spike-induced diffuse alveolar damage in mice (Imai et al., 2005; Kuba et al., 2005; Nicholls and Peiris, 2005). This explains why SARS patients carrying the D allele (deletion involving intron 16) polymorphism of the ACE1 gene, which is associated with higher level of sACE, are more prone to hypoxemia (Itoyama et al., 2004). The D allele, by increasing sACE, results in higher angiotensin II levels and hence susceptibility to injury by SARS-CoV S. ACE2 is protective by inactivating angiotensin II (Imai et al., 2005; Nicholls and Peiris, 2005). Interrupting the renin–angiotensin system protected mice from exacerbation of lung injury aggravated by injection of S (Kuba et al., 2005). These studies showed how viral proteins can tip the balance in normal physiology and cause disease. The therapeutic implications for acute respiratory distress syndrome (ARDS), beyond the respiratory damage caused by SARS-CoV, was noted, but an explanation is needed for the generally mild infections of HCoV-NL63, which also utilizes ACE2 (Nicholls and Peiris, 2005). Perhaps the similarity is limited between human and murine pathophysiology in this infection.

These viral and host factors cannot account for cases showing apparent improvement followed later by deterioration (Peiris et al., 2003a). One school of thought incriminates the innate immune system. Peiris et al. investigated the alveolar macrophages, sentinels of the lower respiratory tract (Cheung et al., 2005). They found that cultured macrophages were infected (as evidenced by translated N protein) but not supportive of SARS-CoV replication. In addition, whereas macrophages infected by HCoV-229E and influenza A (H1N1) produce IFN-β, there is no evidence of induction of IFN-β in SARS-CoV-infected macrophages. Instead, there is elaboration of chemokines CXCL10/IFN-inducible protein 10 (IP-10) and CCL2/monocyte chemotactic protein 1 (MCP-1), possibly explaining aspects of the pathogenesis.

An autoimmune explanation akin to influenza virus-induced Goodpasture syndrome, and dengue hemorrhagic fever and dengue shock syndrome, has been suggested (Lin et al., 2003a). Anti-S2 antibodies were discovered in convalescent sera that cross-reacted with lung epithelial cells. These antibodies were reported to be cytotoxic to type-2 pneumocytes and were not found in non-SARS pneumonia patients (Lin et al., 2005b). Translating this into therapeutic terms, these studies suggest that steroids and plasmapheresis might be tried to correct these pathogenetic mechanisms.

Histologically, the lungs have evidence of patchy ‘‘diffuse’’ alveolar damage with a mixed inflammatory infiltrate, edema, microthrombi (Lang et al., 2003) and hyaline membrane formation (Ding et al., 2003; Franks et al., 2003; Ksiazek et al., 2003; Nicholls et al., 2003). SARS-CoV has been identified within fibrin, macrophages, and type-1 and type-2 pneumocytes by immunohistochemistry (Chan et al., 2005b), *in situ* hybridization (To et al., 2004; Chan et al., 2005b; Xu et al., 2005), and electron microscopy (Nicholls et al., 2003; Shieh et al., 2005). Desquamation of type-1 pneumocytes with proliferation of type-2 pneumocytes were consistently present (Franks et al., 2003; Griffiths et al., 2005). Multinucleated type-2 pneumocytes and macrophages similar to those observed in cell cultures were sometimes found (Franks et al., 2003).

SARS-CoV-infected cells displaying S on the surface fuse with adjacent cells expressing hACE2 (Petit et al., 2005), resulting in a little-appreciated route by which adjacent cells appear to become infected by accretion, even before the release of mature progeny virus. The nuclei may be enlarged and nucleoli prominent, but intranuclear inclusions have not been observed (Ksiazek et al., 2003), consistent with the fact that SARS-CoV replicates in the cytoplasm. When healing begins, it is accompanied by squamous metaplasia (Franks et al., 2003) and fibrosis in some patients. Pneumomediastinum has been reported in 10% of patients, unrelated to intubation or positive pressure ventilation (Peiris et al., 2003a; Chu et al., 2004b).

Extrapulmonary pathology

Although enteric infections by SARS-CoV were common in SARS patients and viral replication in the g.i. tract was documented, morphological changes there were minimal (Leung et al., 2003b; Chan et al., 2005b). In patients with lymphopenia, lymphoid depletion was observed in the splenic white pulp and in mucosa-associated lymphoid tissue of the g.i. tract (Ding et al., 2003; Zhong et al., 2003a; Tse et al., 2004; Gu et al., 2005). Striking hepatic mitotic activity was reported in two patients (Chau et al., 2004). Proof of SARS-CoV infection of the liver, however, has remained elusive (Chan et al., 2005b). Evidence of systemic vasculitis was reported in patients who died in a Chinese hospital (Ding et al., 2003). Recently, immune-mediated orchitis was described in men who died from SARS. Unlike mumps orchitis, which is usually (but not always) unilateral, the involvement was bilateral and appeared to target the germ cells, with the possibility of reduced fertility in men who recovered from the infection (Xu et al., 2005).

Immune response

The innate immune response to SARS-CoV infection included elaboration of acute-phase proteins, chemokines, inflammatory cytokines, and C-type lectins such as MBL. Ip et al. showed that MBL prevents SARS-CoV infection of FRhK-4 cells *in vitro*, in accordance with the non-specific role of this lectin that functions before the antibody response (Ip et al., 2005). They further investigated 569 SARS patients

and demonstrated significantly lower serum levels of MBL and a significant prevalence of low-secretor genetic polymorphism than in 1188 controls. Similar conclusions were drawn in a genetic association study in northern China involving 352 SARS patients and 392 control subjects. Reduced expression of functional MBL was attributed to the codon 54 variant (Zhang et al., 2005b). Leikins et al. showed that the θ-defensin retrocyclin 2 inhibits influenza virus entry into cells by cross-linking surface glycoprotein and that the related MBL and human β-defensin 3 similarly inhibited the step that precedes viral entry (Leikina et al., 2005).

Evidence suggests that adaptive immunity in convalescent patients confers not only lasting protection against SARS-CoV, but also determines the outcome of infection (Zhang et al., 2005c). Convalescent patients develop IgG-class neutralizing antibodies against S protein (Traggiai et al., 2004; Lau and Peiris, 2005; Temperton et al., 2005). These antibodies prevent infection of permissive cell lines *in vitro*. At 7–8 months post-infection, the titer of neutralizing antibody to SARS-CoV remains stable (Traggiai et al., 2004; Chan et al., 2005a), suggesting the potential for lasting immunity and the possibility that developing an effective vaccine might be feasible.

Virologic diagnosis

The identification of SARS-CoV played a crucial role in the development of reliable diagnostic tests. These diagnostic tests were important because viral culture was only available in a few BSL3 laboratories. RT-PCR was developed as soon as SARS-CoV was identified (Drosten et al., 2003a; Ksiazek et al., 2003; Peiris et al., 2003b). At the same time, real-time quantitative reverse transcriptase-PCR (qRT-PCR) assays using intercalating dye, or in conjunction with single or, later, dual TaqMan probes, resulted in a significant increase in sensitivity and throughput compared to conventional RT-PCR (Drosten et al., 2003a; Poon et al., 2003; Jiang et al., 2004; Poon et al., 2004b; Yip et al., 2005). WHO network laboratories made available various primers and protocols for these tests (http://www.who.int/csr/sars/primers/en/index.html). Specimens from the upper respiratory tract, sputum, blood, stool, and urine have all yielded viral nucleic acid at various stages of the illness (Grant et al., 2003; Ng et al., 2003; Tong et al., 2003; Chan et al., 2004d; Hung et al., 2004; Ng et al., 2004). Viral load data generated from qRT-PCR were later shown to correlate with outcome (Chu et al., 2004c; Hung et al., 2004). For laboratories with fewer resources and those concerned about the shelf life of diagnostic kits, a highly sensitive gel-based RT-PCR protocol targeting the proteinase gene has been useful (Inoue et al., 2005).

Tests for antibodies to SARS-CoV, however, were plagued by concerns about sensitivity (only 60% positivity) (Chan et al., 2004b) and specificity. Peiris, Ksiazek, and Drosten used indirect fluorescence antibody (IFA) and cell culture extract-based ELISA assays to demonstrate seroconversion and a progressive increase in antibody titers in SARS (Drosten et al., 2003a; Ksiazek et al., 2003; Peiris et al., 2003b). IFA is specific (Chan et al., 2004c), but labor intensive and has an

additional theoretical risk because virus-infected cells are used on the microscopic slides. ELISA assays using Vero cell extracts may yield false-positive results because of autoantibodies in patients with autoimmune disorders as well as in some normal subjects (Wang et al., 2004b). Recombinant N-based ELISA tests (Woo et al., 2004b) have cross-reacted with other HCoV. Protein immunoblotting against N and S could be used in conjunction with these other assays by identifying the false-positive results (Woo et al., 2004a). Recently, Chan et al. showed that SARS-CoV induces an anamnestic response to HCoV OC43, 229E, and NL63, demanding care in the interpretation of serological findings (Chan et al., 2005a).

Employing Pepscan analyses against convalescent patient sera and vaccine-induced laboratory animal antisera, He et al. identified regions in the M protein (M1-31 and M132-161) unique to SARS-CoV. Recombinant peptides from those regions have been exploited to overcome cross-reactivity in a recently developed ELISA test that correctly discriminated 40 SARS convalescent sera from 30 control sera (He et al., 2005a). Other advances led to the use of pseudotyped virus for the neutralization assay in lieu of SARS-CoV (Giroglou et al., 2004; Han et al., 2004; Yang et al., 2004b; Temperton et al., 2005).

Because of low sensitivity, none of the ‘‘rapid tests’’ can exclude SARS in a suspected case, and the testing of different types of specimens collected at different times is now recommended (Peiris et al., 2003c; Bermingham et al., 2004). The prolonged infectivity of respiratory and stool specimens demand careful handling of specimens (Lai et al., 2005a). Microarray and investigational plasma proteomic approaches to diagnosis [17, 82, 88] are under development. Issues in molecular diagnosis have been reviewed elsewhere (Poon et al., 2004a; Mahony and Richardson, 2005).

Drug therapy

The number of pharmacological agents with a significant selectivity index (CC_{50} divided by EC_{50}) against SARS-CoV has increased as knowledge of SARS-CoV biology has increased (Holmes, 2003). Thomas Lai has reviewed the therapeutic experience during the SARS epidemic (Lai, 2005).

Ribavirin

Ribavirin is a purine nucleoside analog that interacts with viral RNA polymerases as well as having other poorly characterized activities against viruses (Parker, 2005). In therapy of the hepatitis C virus, ribavirin is thought to act by inhibition of GTP synthesis by an effect on inosine monophosphate dehydrogenase, thereby limiting viral RNA synthesis, and by enhancement of Th1 responses, which may assist viral clearance (Thomas et al., 1999). For SARS-CoV, however, its *in vitro* activity is inconsistent (Morgenstern et al., 2005), and it has had no demonstrable clinical benefit in uncontrolled series of patients (Avendano et al., 2003; van Vonderen et al., 2003). In addition to the fact that it is a teratogen, it can cause a

dose-dependent but reversible hemolytic anemia (Knowles et al., 2003). Its continued empirical use in SARS is not recommended (Knowles et al., 2003; van Vonderen et al., 2003).

Other antiviral therapies

Glycyrrhizin (Cinatl et al., 2003) and its derivatives (Wu et al., 2004; Hoever et al., 2005) have shown promising selective activity (selective activity index >33) against SARS-CoV. Its antiviral activities may be related to its effects on cellular signaling pathways, transcription factors (AP-1, NF-kB), and its upregulation of inducible nitrous oxide synthase (Cinatl et al., 2003). Moreover, its effect on lowering plasma membrane fluidity and hence impeding viral entry, is consistent with its observed broad antiviral activity (Harada, 2005).

Chloroquine, discovered by the German chemist Hans Andersag in 1934 and used for the treatment of malaria, amebiasis, HIV, and autoimmune diseases, was recognized to have activity against SARS-CoV *in vitro*, with a selectivity index of 30 against SARS-CoV in Vero E6 cell culture (Savarino et al., 2003; Keyaerts et al., 2004; Vincent et al., 2005). Chloroquine elevates endosomal pH and interferes with terminal glycosylation of ACE2 (Vincent et al., 2005), thus having both non-specific and specific anti-SARS-CoV activities.

Drugs that target viral proteases

Inhibiting SARS-CoV 3CLpro prevents the assembly of a functional replication complex (Bacha et al., 2004; Lee et al., 2005a; Martina et al., 2005). Conservation among coronaviruses suggests that a wide-spectrum inhibitor against the main protease is feasible (Yang et al., 2005a), which may be useful against the several related strains of SARS-CoV-like viruses (Lau et al., 2005; Li et al., 2005c). Homology modeling has revealed remarkable conservation of substrate-binding sites among SARS-CoV, HCoV-229E, and PEDV (Anand et al., 2003). However, one such inhibitor of viral proteases, AG7088, did not show *in vitro* activity at the concentration of $10\,\mu$M (Wu et al., 2004).

During the epidemic in Guangzhou, clinicians observed that HIV-positive patients on highly active anti-retroviral therapy (HAART) appear to be protected against SARS (Chen et al., 2003; Chen and Cao, 2004). In Hong Kong, the utility of lopinavir–ritonavir combination therapy was investigated in a multicenter retrospective matched cohort study as initial and rescue therapy for SARS (Chan et al., 2003). Patients who received this therapy as initial treatment for SARS had better outcome (reduced death and intubation rate) compared with an uncontrolled group, with a lower rate of use of methylprednisolone at a lower mean dose. The results were similar in a subset of those patients reported separately (Chu et al., 2004a). These clinical trials are in agreement with structural studies that predicted the utility of lopinavir, ritonavir, niclosamide, and promazine against 3CL pro

(Zhang and Yap, 2004), with lopinavir and nelfinavir also showing *in vitro* activity (Chen et al., 2004a; Chu et al., 2004a; Yamamoto et al., 2004).

Therapy by blocking viral entry

The interactions between SARS-CoV and host cell involve binding, conformational change of S2, and membrane fusion, all of which are possible targets for therapy. Novel chimeric immunoglobulins such as CD4-IgG (Arthos et al., 2002), have shown benefit in treating HIV-1, and similarly, multivalent sACE2-immunoglobulin might be efficacious against SARS-CoV (Dimitrov, 2003), which can be improved by using residues 90–93 of civet ACE2 (Li et al., 2005e).

Because of the similar mechanism by which SARS-CoV S2 and HIV-1 gp41 mediate viral entry, S protein HR-derived peptides have been predicted to inhibit (Kliger and Levanon, 2003) and later shown to inhibit (Bosch et al., 2004) SARS-CoV infection of Vero cells. Recombinant proteins containing HR1 and HR2 were further shown to have potent inhibitory activities on entry of the HIV/SARS pseudoviruses into cells (Ni et al., 2005). These proteins are cheaper to produce than synthetic peptides and are more stable. Cathepsin L inhibitors could also be studied as entry inhibitors (Simmons et al., 2005).

Monoclonal antibody therapy

Monoclonal antibody with viral neutralizing activity has therapeutic potential against SARS and other viruses (Zhang et al., 2005d) and has been shown to be capable of preventing neonatal respiratory syncytial virus infection (Johnson et al., 1997). Monoclonal antibodies can protect ferrets against SARS-CoV infection (ter Meulen et al., 2004). Convalescent serum has been used in SARS patients, though its efficacy is unknown, and in experimentally infected mice, with possible activity against SARS-CoV infection in the latter (Wong et al., 2003b; Traggiai et al., 2004; Yeh et al., 2005).

An improved B-cell immortalization technique employing a CpG oligonucleotide (CpG 2006) as a polyclonal B-cell activator, was used with Epstein–Barr virus and irradiated allogeneic mononuclear cells to study the B-cell memory of a convalescent SARS patient. Neutralizing antibodies from one stable B-cell clone (S3.1) were found to protect mouse lungs from SARS-CoV challenge (Traggiai et al., 2004). Thirty-five neutralizing monoclonal antibodies were isolated in this study.

Eight recombinant human single-chain variable region fragments (scFvs) against the receptor-binding region of S protein were identified (Sui et al., 2004, 2005). One of these engineered monoclonal antibodies (80R IgG1) showed potent neutralization of SARS-CoV in *in vitro* and animal studies.

Interferons

The IFN signaling pathway is one of the targets of SARS-CoV (Cheung et al., 2005; Law et al., 2005a; Spiegel et al., 2005), suggesting that it may play an important role in host defense against SARS-CoV. Not unexpectedly, type-I IFN (α/β) but not type-II INF (γ) have shown potent inhibition of SARS-CoV infection and replication (Zheng et al., 2004a). Swedish scientists found that natural IFN-α and IFN-β have more potent *in vitro* activity than recombinant IFN-α (Chen et al., 2004b). A CpG oligodeoxynucleotide (BW001) strongly stimulated IFN-α secretion by dendritic cells and peripheral blood mononuclear cells; the supernatant fluid from these cells protected Vero cells from SARS-CoV infection (Bao et al., 2005). Macaques were protected by prophylactic use of pegylated IFN-α (Haagmans et al., 2004). Post-exposure prophylaxis of SARS by IFN yielded intermediate results. Uncontrolled clinical experience with IFN in the treatment of SARS has been reported. However, no randomized double-blind controlled trial has yet been conducted.

Vaccines

No effective vaccine has been developed so far. However, there is some evidence to suggest that successful vaccination may be possible. Molecular emergence studies have shown that SARS-CoV adapts to human ACE2, which presumably is not subject to much evolutionary pressure, and therefore it is likely that SARS-CoV making subsequent jumps to humans from animals will be covered by a vaccine developed against the 2003 human isolate (Lau et al., 2005; Li et al., 2005c; Zhi et al., 2005).

NIAID researchers engineered attenuated bovine-human parainfluenza virus (BHPIV3) to express various SARS-CoV structural proteins (Buchholz et al., 2004). They found that S, but not the other structural proteins (M, E, N), elicited neutralizing antibodies that protected the lungs of hamsters against SARS-CoV (Roberts et al., 2005). When a similar vaccine was given by the nasal route to African green monkeys (*Cercopithecus aethiops*), they shed no virus from the upper respiratory tract after challenge with SARS-CoV (Bukreyev et al., 2004).

Although the receptor-binding region of SARS-CoV is the most important target for neutralizing antibodies, the S2 "region" (Leu 803 to Ala 828), conserved across 45 viral isolates and containing a neutralizing antigenic determinant, was able to elicit neutralizing antibodies that protected some but not all small laboratory mammals against infection with SARS-CoV pseudovirus (Zhang et al., 2004). Over 100 potential cytotoxic T-lymphocyte (CTL) vaccine candidates that cover >99% of all individuals of all major human populations were identified within 6 months of the publication of the virus sequence (Sylvester-Hvid et al., 2004).

Antibody-enhanced viral pathogenicity is generally a concern with coronaviruses (Zhang et al., 2005a). With SARS-CoV infection, ferrets get a more severe hepatitis when first given a vaccinia-based recombinant SARS vaccine (Czub et al., 2005). More recently, NIAID scientists revealed that a pseudovirus expressing a

partially humanized strain S (GD03T13) (GZ-03-01, Fig. 1 of Ref. (Song et al., 2005b) is markedly resistant to neutralization by immune IgG purified from mice vaccinated against S from strains derived from human isolates (Urbani strain) (Yang et al., 2005c). Antibodies raised against the human strain mediated enhanced entry of a lentiviral vector expressing civet S, but resistance to IgG enhancement of civet S is not limited to vaccinated mice; human monoclonal antibodies (S3.1, S111, S117) created from the immune repertoire of a convalescent patient (Traggiai et al., 2004) also exhibited these phenomena. Thus, a vaccine against humanized SARS-CoV might enhance the pathogenicity of a normally low-pathogenic animal SARS-CoV-like virus.

Another study showed that for SARS-CoV, which epitope *not* to employ in a vaccine is important. Interaction of antibody with conformational epitopes in the receptor-binding region was shown to be responsible for antibody-enhanced viral pathogenicity (Yang et al., 2005c). By deleting portions of S (truncated at aa1153), it was seen that the antibodies it induced did not promote enhancement. One monoclonal antibody (S110) was also found that did not mediate enhancement. This is consistent with other studies that have shown cross-reactivity between anti-S2 (aa927–937 and aa942–951) and lung cell antigens (Jiang et al., 2005a; Lin et al., 2005b).

Inactivated SARS-CoV can elicit neutralizing antibody (Takasuka et al., 2004; Tang et al., 2004) and cellular immunity. Mice vaccinated with inactivated SARS-CoV have been protected against challenge with intranasal SARS-CoV (Spruth et al., 2005).

Safety concerns about inactivated SARS-CoV vaccines are those of any inactivated vaccines, and they include the possibility that viral nucleic acid might remain infectious, as in the early days of the inactivated poliovirus vaccine (Katz, 2004; Offit, 2005). Another concern is the autoantibodies induced when Vero cells were used to cultivate SARS-CoV for experimental vaccines; the SARS-CoV neutralizing antibodies cross-reacted with an abundant human serum glycoprotein asialo-orosomucoid (ASOR) (Wang and Lu, 2004). An inactivated SARS-CoV vaccine using alum adjuvant has entered into a clinical trial in China, but the results are not yet available (Enserink, 2004).

The most attenuated SARS-CoV would be a genetically engineered version that could express proteins but that would not be transmissible from cell to cell (Holmes, 2003). Cross-reacting epitopes and toxic viral proteins (Chang et al., 2004; Imai et al., 2005; Kuba et al., 2005) would need to be excised or edited through genetic engineering techniques such as the construction and manipulation of full-length SARS-CoV cDNA (Yount et al., 2002, 2003).

Vectors for such an engineered vaccine could include a weakened version of the human parainfluenza virus 3, called BHPIV3. It is non-invasive (limited to the mucosa) and not known to recombine, unlike the coronaviruses. It replicates efficiently in children and with some engineering, might also replicate in non-naïve adults (Buchholz et al., 2004; Bukreyev et al., 2004), potentially yielding a vaccine with broad applications.

Other possible vectors such as adenovirus have been used to express SARS-CoV genes, and have been used to vaccinate macaque monkeys, eliciting neutralizing antibody and N-specific T-cell response (Gao et al., 2003). A highly attenuated vaccinia virus Ankara modified to express full-length S was given intranasally or intramuscularly to mice; it elicited cross-transferable (to other mice) humoral immunity that protected the upper as well as lower respiratory tracts against SARS-CoV (Bisht et al., 2004). Virus-like particles with no viral nucleic acid but containing S, M, and E proteins have been produced in Taiwan and the UK using insect cells infected by recombinant baculoviruses (Ho et al., 2004; Mortola and Roy, 2004), and might be suitable for study as vaccines.

DNA vaccines have been used to elicit protective humoral and cellular immunity against SARS-CoV in a mouse model (Yang et al., 2004b). A gene-based vaccine has also been tested, boosted by inactivated virus to broaden the immune response (Kong et al., 2005; Talaat and Stemke-Hale, 2005). A combination of nucleocapsid DNA vaccine against SARS-CoV with N protein has been shown to be required to generate antibody and a CD8+ response, and the presence of adjuvant determined whether humoral or cellular immunity is elicited (Azizi et al., 2005). Immunization with plasmid DNA carrying various S fragments have been shown by scientists at Hong Kong University to elicit antibodies in some mice, with the S1 fragment promoting a Th1-mediated antibody isotype switching. Both anti-S1 and anti-S2 antibodies were required for virus neutralization (Zeng et al., 2004a). One study of an experimental DNA vaccine against SARS-CoV showed that N alone was sufficient to induce antibody and CTL responses in mice (Zhu et al., 2004). In another study, N sequences linked to those specifying calreticulin (enhances antigen presentation to CD8+ T-cells) was efficacious in inducing N-specific humoral and cellular immune responses and in reducing the viral titer in mice challenged with N-expressing vaccinia virus (Kim et al., 2004). In mice immunized with plasmids, S, N, and M DNA vaccines were all able to elicit immune responses, with S and M eliciting more potent humoral and cellular immune responses, respectively (Wang et al., 2005c).

Most experience with DNA vaccines in other human viral diseases have been limited to various animal models (Gurunathan et al., 2000), although human studies have begun recently. With careful design, safety could be assured because integration into the genome appears to happen much less often than spontaneous mutations. Targeted delivery of nucleic acids will enable wider application of DNA vaccines (Song et al., 2005a). The ability of DNA vaccines to elicit both cellular and humoral immune responses, and their stability, simplicity, and versatility makes them attractive for immunization against intracellular pathogens such as SARS-CoV. Recent advances in this area have been reviewed (Taylor, 2005).

Implications for the future

A significant public health question is whether SARS-CoV will return. Many infectious diseases have reemerged in recent history (Fauci, 2005). SARS-CoV made

a fleeting reemergence in the winter of 2003–2004 (Liang et al., 2004; Normile, 2004; Song et al., 2005b). With the discovery that the bat is the natural reservoir, it appears likely that SARS-CoV will eventually reemerge. However, the prospect of another large outbreak is low because an animal strain would have to adapt to humans before becoming epidemic, although it is not impossible that human isolate of SARS-CoV could have escaped back to the wild. Human strains of SARS-CoV bind efficiently to civet ACE2 (Li et al., 2005e). It might also bind bat ACE2 with avidity. The ease of species jumping as humans move into areas with SARS-CoV is suggested by the finding of SARS-CoV in rats, and the identification of serologically positive civets raised for the pet market in a farm in Shanwei, Guangdong. In the latter case, seed animals for that farm were bought at various markets in the province (Tu et al., 2004). Bats, civets, rodents, and other small mammals will need to be included in the surveillance for the presence of SARS-CoV.

Molecular emergence studies showed that most of the adaptations required for humanization of SARS-CoV took place within a short span early in the epidemic. This indicates a narrow window of opportunity for prevention of reemergence of SARS or the emergence of other viral infections. We missed the opportunity to prevent the emergence of SARS but managed to limit the damage. The conditions that can lead to reemergence are over-development and habitat destruction, excessive civet consumption, poor hygiene in markets and farms, etc.

Because poor market hygiene and management were major factors in the recent epidemic, major effort should be made to rectify the situation. Culinary culture needs to be modified in the interest of public health. Civets have been removed from the menu in Guandong and should remain so. Animals raised for food must be provided care and veterinary service, and wild animals should be left alone. Anthropocentric modification of the natural environment can lead to serious consequences (Dobson, 2005).

The careful design of hospitals (Li et al., 2005g), housing projects, and public places, with the unseen microbe in mind and with a view of providing clean breathing air will bring about the next quantum leap in public health (Tong and Liang, 2004; Tong, 2005a). Within health care facilities, awareness of infection control should be regularly refreshed so as to convert health care workers from "vectors" to infection controllers (Lloyd-Smith et al., 2003).

Amidst the exciting progress in vaccine development and drug discovery, we should be mindful that vaccines seldom achieve perfect immunity, and drug resistance is to be expected. However, the best time to face up to an emerging infectious disease is "now"—even before the outbreaks occur (Zhong et al., 2003b; Kuiken et al., 2005). Vaccines and drugs would be in the second line of defense, with public health measures being the first. Prevention of viral adaptation to the human receptor as a strategy against emerging viral diseases is the single most important translation from molecular emergence studies to public health management.

Last but not least, physicians must serve as sentinels for danger from this virus (Reilley et al., 2003). The lessons learned from this epidemic reinforced the need to

strengthen surveillance and international cooperation, to maintain/upgrade time-honored infectious disease control practices, and to invest in rapid response capabilities such as the WHO. A paradigm shift to a better balance between defense (treatment) and preempting (prevention) is in order.

References

Anon., Cluster of severe acute respiratory syndrome cases among protected health-care workers—Toronto, Canada, April 2003. MMWR Morb Mortal Wkly Rep 2003a; 52: 433–436.

Anon., A multicentre collaboration to investigate the cause of severe acute respiratory syndrome. Lancet 2003b; 361: 1730–1733.

Anon., Prevalence of IgG antibody to SARS-associated coronavirus in animal traders—Guangdong Province, China, 2003. MMWR Morb Mortal Wkly Rep 2003c; 52: 986–987.

Anon., Update: outbreak of severe acute respiratory syndrome—worldwide, 2003. MMWR Morb Mortal Wkly Rep 2003d; 52: 269–272.

Anand K, Ziebuhr J, Wadhwani P, Mesters JR, Hilgenfeld R. Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs. Science 2003; 300: 1763–1767.

Anderson LJ. Infectious diseases: escaping an epidemic. Science 2005; 310: 444–445.

Arthos J, Cicala C, Steenbeke TD, Chun TW, Dela Cruz C, Hanback DB, Khazanie P, Nam D, Schuck P, Selig SM, Van Ryk D, Chaikin MA, Fauci AS. Biochemical and biological characterization of a dodecameric CD4-Ig fusion protein: implications for therapeutic and vaccine strategies. J Biol Chem 2002; 277: 11456–11464.

Avendano M, Derkach P, Swan S. Clinical course and management of SARS in health care workers in Toronto: a case series. CMAJ 2003; 168: 1649–1660.

Azizi A, Aucoin S, Ghorbani M, Soare C, Naas T, Tadesse H, Frost R, Diaz-Mitoma F. A combined nucleocapsid vaccine induces vigorous SARS-CD8+ T-cell immune responses. Genet Vaccines Ther 2005; 3: 7.

Bacha U, Barrila J, Velazquez-Campoy A, Leavitt SA, Freire E. Identification of novel inhibitors of the SARS coronavirus main protease 3CLpro. Biochemistry 2004; 43: 4906–4912.

Bao M, Zhang Y, Wan M, Dai L, Hu X, Wu X, Wang L, Deng P, Wang J, Chen J, Liu Y, Yu Y, Wang L. Anti-SARS-CoV immunity induced by a novel CpG oligodeoxynucleotide. Clin. Immunol. 2005; 118: 180–187.

Bergeron E, Vincent MJ, Wickham L, Hamelin J, Basak A, Nichol ST, Chretien M, Seidah NG. Implication of proprotein convertases in the processing and spread of severe acute respiratory syndrome coronavirus. Biochem Biophys Res Commun 2005; 326: 554–563.

Bermingham A, Heinen P, Iturriza-Gomara M, Gray J, Appleton H, Zambon MC. Laboratory diagnosis of SARS. Philos Trans R Soc Lond B Biol Sci 2004; 359: 1083–1089.

Bisht H, Roberts A, Vogel L, Bukreyev A, Collins PL, Murphy BR, Subbarao K, Moss B. Severe acute respiratory syndrome coronavirus spike protein expressed by attenuated vaccinia virus protectively immunizes mice. Proc Natl Acad Sci USA 2004; 101: 6641–6646.

Booth CM, Matukas LM, Tomlinson GA, Rachlis AR, Rose DB, Dwosh HA, Walmsley SL, Mazzulli T, Avendano M, Derkach P, Ephtimios IE, Kitai I, Mederski BD,

Shadowitz SB, Gold WL, Hawryluck LA, Rea E, Chenkin JS, Cescon DW, Poutanen SM, Detsky AS. Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area. JAMA 2003; 289: 2801–2809.

Booth TF, Kournikakis B, Bastien N, Ho J, Kobasa D, Stadnyk L, Li Y, Spence M, Paton S, Henry B, Mederski B, White D, Low DE, McGeer A, Simor A, Vearncombe M, Downey J, Jamieson FB, Tang P, Plummer F. Detection of airborne severe acute respiratory syndrome (SARS) coronavirus and environmental contamination in SARS outbreak units. J Infect Dis 2005; 191: 1472–1477.

Bosch BJ, Martina BE, Van Der Zee R, Lepault J, Haijema BJ, Versluis C, Heck AJ, De Groot R, Osterhaus AD, Rottier PJ. Severe acute respiratory syndrome coronavirus (SARS-CoV) infection inhibition using spike protein heptad repeat-derived peptides. Proc Natl Acad Sci USA 2004; 101: 8455–8460.

Breslin JJ, Mork I, Smith MK, Vogel LK, Hemmila EM, Bonavia A, Talbot PJ, Sjostrom H, Noren O, Holmes KV. Human coronavirus 229E: receptor binding domain and neutralization by soluble receptor at 37 degrees C. J Virol 2003; 77: 4435–4438.

Buchholz UJ, Bukreyev A, Yang L, Lamirande EW, Murphy BR, Subbarao K, Collins PL. Contributions of the structural proteins of severe acute respiratory syndrome coronavirus to protective immunity. Proc Natl Acad Sci USA 2004; 101: 9804–9809.

Bukreyev A, Lamirande EW, Buchholz UJ, Vogel LN, Elkins WR, St Claire M, Murphy BR, Subbarao K, Collins PL. Mucosal immunisation of African green monkeys (*Cercopithecus aethiops*) with an attenuated parainfluenza virus expressing the SARS coronavirus spike protein for the prevention of SARS. Lancet 2004; 363: 2122–2127.

Cai CZ, Han LY, Chen X, Cao ZW, Chen YZ. Prediction of functional class of the SARS coronavirus proteins by a statistical learning method. J Proteome Res 2005; 4: 1855–1862.

Campanacci V, Egloff MP, Longhi S, Ferron F, Rancurel C, Salomoni A, Durousseau C, Tocque F, Bremond N, Dobbe JC, Snijder EJ, Canard B, Cambillau C. Structural genomics of the SARS coronavirus: cloning, expression, crystallization and preliminary crystallographic study of the Nsp9 protein. Acta Crystallogr D Biol Crystallogr 2003; 59: 1628–1631.

Cavanagh D. Nidovirales: a new order comprising *Coronaviridae* and *Arteriviridae*. Arch Virol 1997; 142: 629–633.

Chan CW, Chiu WK, Chan CC, Chow EY, Cheung HM, Ip PL. Osteonecrosis in children with severe acute respiratory syndrome. Pediatr Infect Dis J 2004a; 23: 888–890.

Chan KH, Cheng VCC, Woo PCY, Lau SKP, Poon LLM, Guan Y, Seto WH, Yuen KY, Peiris JSM. Serological responses in patients with severe acute respiratory syndrome coronavirus infection and cross-reactivity with human coronaviruses 229E, OC43, and NL63. Clin Diagn Lab Immunol 2005a; 12: 1317–1321.

Chan KH, Poon LL, Cheng VC, Guan Y, Hung IF, Kong J, Yam LY, Seto WH, Yuen KY, Peiris JS. Detection of SARS coronavirus in patients with suspected SARS. Emerg Infect Dis 2004b; 10: 294–299.

Chan KS, Lai ST, Chu CM, Tsui E, Tam CY, Wong MM, Tse MW, Que TL, Peiris JS, Sung J, Wong VC, Yuen KY. Treatment of severe acute respiratory syndrome with lopinavir/ritonavir: a multicentre retrospective matched cohort study. Hong Kong Med J 2003; 9: 399–406.

Chan PK, Ng KC, Chan RC, Lam RK, Chow VC, Hui M, Wu A, Lee N, Yap FH, Cheng FW, Sung JJ, Tam JS. Immunofluorescence assay for serologic diagnosis of SARS. Emerg Infect Dis 2004c; 10: 530–532.

Chan PK, To WK, Ng KC, Lam RK, Ng TK, Chan RC, Wu A, Yu WC, Lee N, Hui DS, Lai ST, Hon EK, Li CK, Sung JJ, Tam JS. Laboratory diagnosis of SARS. Emerg Infect Dis 2004d; 10: 825–831.

Chan WS, Wu C, Chow SC, Cheung T, To KF, Leung WK, Chan PK, Lee KC, Ng HK, Au DM, Lo AW. Coronaviral hypothetical and structural proteins were found in the intestinal surface enterocytes and pneumocytes of severe acute respiratory syndrome (SARS). Mod. Pathol. 2005; 18: 1432–1439.

Chandran K, Sullivan NJ, Felbor U, Whelan SP, Cunningham JM. Endosomal proteolysis of the Ebola virus glycoprotein is necessary for infection. Science 2005; 308: 1643–1645.

Chang CK, Sue SC, Yu TH, Hsieh CM, Tsai CK, Chiang YC, Lee SJ, Hsiao HH, Wu WJ, Chang WL, Lin CH, Huang TH. Modular organization of SARS coronavirus nucleocapsid protein. J. Biomed Sci. 2005; 13: 59–72.

Chang YJ, Liu CY, Chiang BL, Chao YC, Chen CC. Induction of IL-8 release in lung cells via activator protein-1 by recombinant baculovirus displaying severe acute respiratory syndrome-coronavirus spike proteins: identification of two functional regions. J Immunol 2004; 173: 7602–7614.

Chau TN, Lee KC, Yao H, Tsang TY, Chow TC, Yeung YC, Choi KW, Tso YK, Lau T, Lai ST, Lai CL. SARS-associated viral hepatitis caused by a novel coronavirus: report of three cases. Hepatology 2004; 39: 302–310.

Chen F, Chan KH, Jiang Y, Kao RY, Lu HT, Fan KW, Cheng VC, Tsui WH, Hung IF, Lee TS, Guan Y, Peiris JS, Yuen KY. *In vitro* susceptibility of 10 clinical isolates of SARS coronavirus to selected antiviral compounds. J Clin Virol 2004a; 31: 69–75.

Chen L, Liu P, Gao H, Sun B, Chao D, Wang F, Zhu Y, Hedenstierna G, Wang CG. Inhalation of nitric oxide in the treatment of severe acute respiratory syndrome: a rescue trial in Beijing. Clin Infect Dis 2004b; 39: 1531–1535.

Chen RF, Chang JC, Yeh WT, Lee CH, Liu JW, Eng HL, Yang KD. Role of vascular cell adhesion molecules and leukocyte apoptosis in the lymphopenia and thrombocytopenia of patients with severe acute respiratory syndrome (SARS). Microbes Infect. 2005; 8: 122–127.

Chen W, Yan M, Yang L, Ding B, He B, Wang Y, Liu X, Liu C, Zhu H, You B, Huang S, Zhang J, Mu F, Xiang Z, Feng X, Wen J, Fang J, Yu J, Yang H, Wang J. SARS-associated coronavirus transmitted from human to pig. Emerg Infect Dis 2005b; 11: 446–448.

Chen XP, Cao Y. Consideration of highly active antiretroviral therapy in the prevention and treatment of severe acute respiratory syndrome. Clin Infect Dis 2004; 38: 1030–1032.

Chen XP, Li GH, Tang XP, Xiong Y, Chen XJ, Cao Y. Lack of severe acute respiratory syndrome in 19 AIDS patients hospitalized together. J Acquir Immune Defic Syndr 2003; 34: 242–243.

Cheng PK, Wong DA, Tong LK, Ip SM, Lo AC, Lau CS, Yeung EY, Lim WW. Viral shedding patterns of coronavirus in patients with probable severe acute respiratory syndrome. Lancet 2004; 363: 1699–1700.

Cheung CY, Poon LL, Ng IH, Luk W, Sia SF, Wu MH, Chan KH, Yuen KY, Gordon S, Guan Y, Peiris JS. Cytokine responses in severe acute respiratory syndrome coronavirus-infected macrophages *in vitro*: possible relevance to pathogenesis. J Virol 2005; 79: 7819–7826.

Chinese SMEC. Molecular evolution of the SARS coronavirus during the course of the SARS epidemic in China. Science 2004; 303: 1666–1669.

Choi KW, Chau TN, Tsang O, Tso E, Chiu MC, Tong WL, Lee PO, Ng TK, Ng WF, Lee KC, Lam W, Yu WC, Lai JY, Lai ST. Outcomes and prognostic factors in 267 patients with severe acute respiratory syndrome in Hong Kong. Ann Intern Med 2003; 139: 715–723.

Chow KY, Yeung YS, Hon CC, Zeng F, Law KM, Leung FC. Adenovirus-mediated expression of the C-terminal domain of SARS-CoV spike protein is sufficient to induce apoptosis in Vero E6 cells. FEBS Lett. 2005; 579: 6699–6704.

Chowell G, Castillo-Chavez C, Fenimore PW, Kribs-Zaleta CM, Arriola L, Hyman JM. Model parameters and outbreak control for SARS. Emerg Infect Dis 2004; 10: 1258–1263.

Christian MD, Poutanen SM, Loutfy MR, Muller MP, Low DE. Severe acute respiratory syndrome. Clin Infect Dis 2004; 38: 1420–1427.

Chu CM, Cheng VC, Hung IF, Wong MM, Chan KH, Chan KS, Kao RY, Poon LL, Wong CL, Guan Y, Peiris JS, Yuen KY. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. Thorax 2004a; 59: 252–256.

Chu CM, Leung YY, Hui JY, Hung IF, Chan VL, Leung WS, Law KI, Chan CS, Chan KS, Yuen KY. Spontaneous pneumomediastinum in patients with severe acute respiratory syndrome. Eur Respir J 2004b; 23: 802–804.

Chu CM, Poon LL, Cheng VC, Chan KS, Hung IF, Wong MM, Chan KH, Leung WS, Tang BS, Chan VL, Ng WL, Sim TC, Ng PW, Law KI, Tse DM, Peiris JS, Yuen KY. Initial viral load and the outcomes of SARS. CMAJ 2004c; 171: 1349–1352.

Chung GT, Chiu RW, Cheung JL, Jin Y, Chim SS, Chan PK, Lo YD. A simple and rapid approach for screening of SARS-coronavirus genotypes: an evaluation study. BMC Infect Dis 2005; 5: 87.

Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. Lancet 2003; 361: 2045–2046.

Crotty S, Cameron CE, Andino R. RNA virus error catastrophe: direct molecular test by using ribavirin. Proc Natl Acad Sci USA 2001; 98: 6895–6900.

Czub M, Weingartl H, Czub S, He R, Cao J. Evaluation of modified vaccinia virus Ankara based recombinant SARS vaccine in ferrets. Vaccine 2005; 23: 2273–2279.

Dimitrov DS. The secret life of ACE2 as a receptor for the SARS virus. Cell 2003; 115: 652–653.

Ding Y, He L, Zhang Q, Huang Z, Che X, Hou J, Wang H, Shen H, Qiu L, Li Z, Geng J, Cai J, Han H, Li X, Kang W, Weng D, Liang P, Jiang S. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. J Pathol 2004; 203: 622–630.

Ding Y, Wang H, Shen H, Li Z, Geng J, Han H, Cai J, Li X, Kang W, Weng D, Lu Y, Wu D, He L, Yao K. The clinical pathology of severe acute respiratory syndrome (SARS): a report from China. J Pathol 2003; 200: 282–289.

Dobson AP. Virology: what links bats to emerging infectious diseases? Science 2005; 310: 628–629.

Donoghue M, HsiehF, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breitbart RE, Acton S. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circ Res 2000; 87: E1–E9.

Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Becker S, Rabenau H, Panning M, Kolesnikova L, Fouchier RA, Berger A, Burguiere AM, Cinatl J, Eickmann M,

Escriou N, Grywna K, Kramme S, Manuguerra JC, Muller S, Rickerts V, Sturmer M, Vieth S, Klenk HD, Osterhaus AD, Schmitz H, Doerr HW. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 2003a; 348: 1967–1976.

Drosten C, Preiser W, Gunther S, Schmitz H, Doerr HW. Severe acute respiratory syndrome: identification of the etiological agent. Trends Mol Med 2003b; 9: 325–327.

Edwards DA, Man JC, Brand P, Katstra JP, Sommerer K, Stone HA, Nardell E, Scheuch G. Inhaling to mitigate exhaled bioaerosols. Proc Natl Acad Sci USA 2004; 101: 17383–17388.

Egloff MP, Ferron F, Campanacci V, Longhi S, Rancurel C, Dutartre H, Snijder EJ, Gorbalenya AE, Cambillau C, Canard B. The severe acute respiratory syndrome-coronavirus replicative protein nsp9 is a single-stranded RNA-binding subunit unique in the RNA virus world. Proc Natl Acad Sci USA 2004; 101: 3792–3796.

Eigen M. New concepts for dealing with the evolution of nucleic acids. Cold Spring Harb Symp Quant Biol 1987; 52: 307–320.

Emeny JM, Morgan MJ. Regulation of the interferon system: evidence that Vero cells have a genetic defect in interferon production. J Gen Virol 1979; 43: 247–252.

Enserink M. Albert Osterhaus profile. The virus collector. Science 2003a; 300: 1228–1229.

Enserink M. Breakthrough of the year. SARS: a pandemic prevented. Science 2003b; 302: 2045.

Enserink M. SARS in China. The big question now: will it be back? Science 2003c; 301: 299.

Enserink M. Infectious diseases. One year after outbreak, SARS virus yields some secrets. Science 2004; 304: 1097.

Fang X, Ye L, Timani KA, Li S, Zen Y, Zhao M, Zheng H, Wu Z. Peptide domain involved in the interaction between membrane protein and nucleocapsid protein of SARS-associated coronavirus. J Biochem Mol Biol 2005; 38: 381–385.

Fauci AS. Emerging and reemerging infectious diseases: the perpetual challenge. Acad Med 2005; 80: 1079–1085.

Fouchier RA, Kuiken T, Schutten M, van Amerongen G, van Doornum GJ, van den Hoogen BG, Peiris M, Lim W, Stohr K, Osterhaus AD. Aetiology: Koch's postulates fulfilled for SARS virus. Nature 2003; 423: 240.

Fowler RA, Scales DC, Ilan R. Evidence of airborne transmission of SARS. N Engl J Med 2004; 351: 609–611.

Franks TJ, Chong PY, Chui P, Galvin JR, Lourens RM, Reid AH, Selbs E, McEvoy CP, Hayden CD, Fukuoka J, Taubenberger JK, Travis WD. Lung pathology of severe acute respiratory syndrome (SARS): a study of 8 autopsy cases from Singapore. Hum Pathol 2003; 34: 743–748.

Galvani AP, May RM. Epidemiology: dimensions of superspreading. Nature 2005; 438: 293–295.

Gao W, Tamin A, Soloff A, D'Aiuto L, Nwanegbo E, Robbins PD, Bellini WJ, Barratt-Boyes S, Gambotto A. Effects of a SARS-associated coronavirus vaccine in monkeys. Lancet 2003; 362: 1895–1896.

Giroglou T, Cinatl Jr. J, Rabenau H, Drosten C, Schwalbe H, Doerr HW, von Laer D. Retroviral vectors pseudotyped with severe acute respiratory syndrome coronavirus S protein. J Virol 2004; 78: 9007–9015.

Gorbalenya AE, Snijder EJ, Spaan WJ. Severe acute respiratory syndrome coronavirus phylogeny: toward consensus. J Virol 2004; 78: 7863–7866.

Graham RL, Sims AC, Brockway SM, Baric RS, Denison MR. The nsp2 replicase proteins of murine hepatitis virus and severe acute respiratory syndrome coronavirus are dispensable for viral replication. J Virol 2005; 79: 13399–13411.

Gramberg T, Hofmann H, Moller P, Lalor PF, Marzi A, Geier M, Krumbiegel M, Winkler T, Kirchhoff F, Adams DH, Becker S, Munch J, Pohlmann S. LSECtin interacts with filovirus glycoproteins and the spike protein of SARS coronavirus. Virology 2005; 340: 224–236.

Grant PR, Garson JA, Tedder RS, Chan PK, Tam JS, Sung JJ. Detection of SARS coronavirus in plasma by real-time RT-PCR. N Engl J Med 2003; 349: 2468–2469.

Griffith JF, Antonio GE, Kumta SM, Hui DS, Wong JK, Joynt GM, Wu AK, Cheung AY, Chiu KH, Chan KM, Leung PC, Ahuja AT. Osteonecrosis of hip and knee in patients with severe acute respiratory syndrome treated with steroids. Radiology 2005; 235: 168–175.

Griffiths MJ, Bonnet D, Janes SM. Stem cells of the alveolar epithelium. Lancet 2005; 366: 249–260.

Groneberg DA, Hilgenfeld R, Zabel P. Molecular mechanisms of severe acute respiratory syndrome (SARS). Respir Res 2005; 6: 8.

Gu J, Gong E, Zhang B, Zheng J, Gao Z, Zhong Y, Zou W, Zhan J, Wang S, Xie Z, Zhuang H, Wu B, Zhong H, Shao H, Fang W, Gao D, Pei F, Li X, He Z, Xu D, Shi X, Anderson VM, Leong ASY. Multiple organ infection and the pathogenesis of SARS. J Exp Med 2005; 202: 415–424.

Guan Y, Peiris JS, Zheng B, Poon LL, Chan KH, Zeng FY, Chan CW, Chan MN, Chen JD, Chow KY, Hon CC, Hui KH, Li J, Li VY, Wang Y, Leung SW, Yuen KY, Leung FC. Molecular epidemiology of the novel coronavirus that causes severe acute respiratory syndrome. Lancet 2004; 363: 99–104.

Guan Y, Zheng BJ, He YQ, Liu XL, Zhuang ZX, Cheung CL, Luo SW, Li PH, Zhang LJ, Guan YJ, Butt KM, Wong KL, Chan KW, Lim W, Shortridge KF, Yuen KY, Peiris JS, Poon LL. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science 2003; 302: 276–278.

Guarino LA, Bhardwaj K, Dong W, Sun J, Holzenburg A, Kao C. Mutational analysis of the SARS virus Nsp15 endoribonuclease: identification of residues affecting hexamer formation. J Mol Biol. 2005; 353: 1106–1117.

Guillen J, Perez-Berna AJ, Moreno MR, Villalain J. Identification of the membrane-active regions of the severe acute respiratory syndrome coronavirus spike membrane glycoprotein using a 16/18-mer peptide scan: implications for the viral fusion mechanism. J Virol 2005; 79: 1743–1752.

Gurunathan S, Klinman DM, Seder RA. DNA vaccines: immunology, application, and optimization. Annu Rev Immunol 2000; 18: 927–974.

Gutierrez AL, Denova-Ocampo M, Racaniello VR, del Angel RM. Attenuating mutations in the poliovirus 5′ untranslated region alter its interaction with polypyrimidine tract-binding protein. J Virol 1997; 71: 3826–3833.

Haagmans BL, Kuiken T, Martina BE, Fouchier RA, Rimmelzwaan GF, van Amerongen G, van Riel D, de Jong T, Itamura S, Chan KH, Tashiro M, Osterhaus AD. Pegylated interferon-alpha protects type 1 pneumocytes against SARS coronavirus infection in macaques. Nat Med 2004; 10: 290–293.

Hamming I, Timens W, Bulthuis ML, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 2004; 203: 631–637.

Han DP, Kim HG, Kim YB, Poon LL, Cho MW. Development of a safe neutralization assay for SARS-CoV and characterization of S-glycoprotein. Virology 2004; 326: 140–149.

Harada S. Broad anti-viral agent glycyrrhizin directly modulates the fluidity of plasma membrane and HIV-1 envelope. Biochem J 2005; 392: 191–199.

Harmer D, Gilbert M, Borman R, Clark KL. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. FEBS Lett 2002; 532: 107–110.

He R, Leeson A, Andonov A, Li Y, Bastien N, Cao J, Osiowy C, Dobie F, Cutts T, Ballantine M, Li X. Activation of AP-1 signal transduction pathway by SARS coronavirus nucleocapsid protein. Biochem Biophys Res Commun 2003; 311: 870–876.

He R, Leeson A, Ballantine M, Andonov A, Baker L, Dobie F, Li Y, Bastien N, Feldmann H, Strocher U, Theriault S, Cutts T, Cao J, Booth TF, Plummer FA, Tyler S, Li X. Characterization of protein–protein interactions between the nucleocapsid protein and membrane protein of the SARS coronavirus. Virus Res 2004a; 105: 121–125.

He Y, Zhou Y, Siddiqui P, Jiang S. Inactivated SARS-CoV vaccine elicits high titers of spike protein-specific antibodies that block receptor binding and virus entry. Biochem Biophys Res Commun 2004b; 325: 445–452.

He Y, Zhou Y, Siddiqui P, Niu J, Jiang S. Identification of immunodominant epitopes on the membrane protein of the severe acute respiratory syndrome-associated coronavirus. J Clin Microbiol 2005a; 43: 3718–3726.

He Y, Zhou Y, Wu H, Luo B, Chen J, Li W, Jiang S. Identification of immunodominant sites on the spike protein of severe acute respiratory syndrome (SARS) coronavirus: implication for developing SARS diagnostics and vaccines. J Immunol 2004c; 173: 4050–4057.

He Z, Zhao C, Dong Q, Zhuang H, Song S, Peng G, Dwyer DE. Effects of acute severe acute respiratory syndrome (SARS) coronavirus infection on peripheral blood lymphocytes and their subsets. Int J Infect Dis. 2005; 9: 323–330.

Heymann D. David Heymann—WHO's public health guru. Interview by Haroon Ashraf. Lancet Infect Dis 2004; 4: 785–788.

Ho Y, Lin PH, Liu CY, Lee SP, Chao YC. Assembly of human severe acute respiratory syndrome coronavirus-like particles. Biochem Biophys Res Commun 2004; 318: 833–838.

Hoever G, Baltina L, Michaelis M, Kondratenko R, Baltina L, Tolstikov GA, Doerr HW, Cinatl Jr. J. Antiviral activity of glycyrrhizic acid derivatives against SARS-coronavirus. J Med Chem 2005; 48: 1256–1259.

Hofmann H, Geier M, Marzi A, Krumbiegel M, Peipp M, Fey GH, Gramberg T, Pohlmann S. Susceptibility to SARS coronavirus S protein-driven infection correlates with expression of angiotensin converting enzyme 2 and infection can be blocked by soluble receptor. Biochem Biophys Res Commun 2004; 319: 1216–1221.

Hofmann H, Pyrc K, van der Hoek L, Geier M, Berkhout B, Pohlmann S. Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. Proc Natl Acad Sci USA 2005; 102: 7988–7993.

Holmes EC, Rambaut A. Viral evolution and the emergence of SARS coronavirus. Philos Trans R Soc Lond B Biol Sci 2004; 359: 1059–1065.

Holmes KV. SARS coronavirus: a new challenge for prevention and therapy. J Clin Invest 2003; 111: 1605–1609.

Holmes KV. Structural biology. Adaptation of SARS coronavirus to humans. Science 2005; 309: 1822–1823.

Hon KL, Leung CW, Cheng WT, Chan PK, Chu WC, Kwan YW, Li AM, Fong NC, Ng PC, Chiu MC, Li CK, Tam JS, Fok TF. Clinical presentations and outcome of severe acute respiratory syndrome in children. Lancet 2003; 361: 1701–1703.

Horimoto T, Kawaoka Y. Influenza: lessons from past pandemics, warnings from current incidents. Nat Rev Microbiol 2005; 3: 591–600.

Hsieh P-K, Chang SC, Huang C-C, Lee T-T, Hsiao C-W, Kou Y-H, Chen IY, Chang C-K, Huang T-H, Chang M-F. Assembly of severe acute respiratory syndrome coronavirus RNA packaging signal into virus-like particles is nucleocapsid dependent. J Virol 2005; 79: 13848–13855.

Hui DS, Wong KT, Ko FW, Tam LS, Chan DP, Woo J, Sung JJY. The 1-year impact of severe acute respiratory syndrome on pulmonary function, exercise capacity, and quality of life in a cohort of survivors. Chest 2005; 128: 2247–2261.

Hung IF, Cheng VC, Wu AK, Tang BS, Chan KH, Chu CM, Wong MM, Hui WT, Poon LL, Tse DM, Chan KS, Woo PC, Lau SK, Peiris JS, Yuen KY. Viral loads in clinical specimens and SARS manifestations. Emerg Infect Dis 2004; 10: 1550–1557.

Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, Crackower MA, Fukamizu A, Hui CC, Hein L, Uhlig S, Slutsky AS, Jiang C, Penninger JM. Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature 2005; 436: 112–116.

Inoue M, Barkham T, Keong LK, Gee LS, Wanjin H. Performance of single-step gel-based reverse transcription-PCR (RT-PCR) assays equivalent to that of real-time RT-PCR assays for detection of the severe acute respiratory syndrome-associated coronavirus. J Clin Microbiol 2005; 43: 4262–4265.

Ip WK, Chan KH, Law HK, Tso GH, Kong EK, Wong WH, To YF, Yung RW, Chow EY, Au KL, Chan EY, Lim W, Jensenius JC, Turner MW, Peiris JS, Lau YL. Mannose-binding lectin in severe acute respiratory syndrome coronavirus infection. J Infect Dis 2005; 191: 1697–1704.

Ito N, Mossel EC, Narayanan K, Popov VL, Huang C, Inoue T, Peters CJ, Makino S. Severe acute respiratory syndrome coronavirus 3a protein is a viral structural protein. J Virol 2005; 79: 3182–3186.

Itoyama S, Keicho N, Quy T, Phi NC, Long HT, Ha le D, Ban VV, Ohashi J, Hijikata M, Matsushita I, Kawana A, Yanai H, Kirikae T, Kuratsuji T, Sasazuki T. ACE1 polymorphism and progression of SARS. Biochem Biophys Res Commun 2004; 323: 1124–1129.

Jeffers SA, Tusell SM, Gillim-Ross L, Hemmila EM, Achenbach JE, Babcock GJ, Thomas Jr. WD, Thackray LB, Young MD, Mason RJ, Ambrosino DM, Wentworth DE, Demartini JC, Holmes KV. CD209L (L-SIGN) is a receptor for severe acute respiratory syndrome coronavirus. Proc Natl Acad Sci USA 2004; 101: 15748–15753.

Jia HP, Look DC, Shi L, Hickey M, Pewe L, Netland J, Farzan M, Wohlford-Lenane C, Perlman S, McCray Jr PB. ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia. J Virol 2005; 79: 14614–14621.

Jiang S, He Y, Liu S. SARS vaccine development. Emerg Infect Dis 2005a; 11: 1016–1020.

Jiang SS, Chen TC, Yang JY, Hsiung CA, Su IJ, Liu YL, Chen PC, Juang JL. Sensitive and quantitative detection of severe acute respiratory syndrome coronavirus infection by real-time nested polymerase chain reaction. Clin Infect Dis 2004; 38: 293–296.

Jiang XS, Tang LY, Dai J, Zhou H, Li SJ, Xia QC, Wu JR, Zeng R. Quantitative analysis of SARS-coronavirus infected cells using proteomic approaches: implications for cellular responses to virus infection. Mol Cell Proteomics 2005; 4: 902–913.

Johnson S, Oliver C, Prince GA, Hemming VG, Pfarr DS, Wang SC, Dormitzer M, O'Grady J, Koenig S, Tamura JK, Woods R, Bansal G, Couchenour D, Tsao E, Hall WC, Young JF. Development of a humanized monoclonal antibody (MEDI-493) with potent *in vitro* and *in vivo* activity against respiratory syncytial virus. J Infect Dis 1997; 176: 1215–1224.

Kan B, Wang M, Jing H, Xu H, Jiang X, Yan M, Liang W, Zheng H, Wan K, Liu Q, Cui B, Xu Y, Zhang E, Wang H, Ye J, Li G, Li M, Cui Z, Qi X, Chen K, Du L, Gao K, Zhao Y-t, Zou X-z, Feng Y-J, Gao Y-F, Hai R, Yu D, Guan Y, Xu J. Molecular evolution analysis and geographic investigation of severe acute respiratory syndrome coronavirus-like virus in palm civets at an animal market and on farms. J Virol 2005; 79: 11892–11900.

Kang X, Xu Y, Wu X, Liang Y, Wang C, Guo J, Wang Y, Chen M, Wu D, Wang Y, Bi S, Qiu Y, Lu P, Cheng J, Xiao B, Hu L, Gao X, Liu J, Wang Y, Song Y, Zhang L, Suo F, Chen T, Huang Z, Zhao Y, Lu H, Pan C, Tang H. Proteomic fingerprints for potential application to early diagnosis of severe acute respiratory syndrome. Clin Chem 2005; 51: 56–64.

Katz SL. From culture to vaccine—Salk and Sabin. N Engl J Med 2004; 351: 1485–1487.

Keyaerts E, Vijgen L, Maes P, Neyts J, Van Ranst M. *In vitro* inhibition of severe acute respiratory syndrome coronavirus by chloroquine. Biochem Biophys Res Commun 2004; 323: 264–268.

Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, Crowley D, Bronson RT, Jacks T. Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell 2005; 121: 823–835.

Kim TW, Lee JH, Hung CF, Peng S, Roden R, Wang MC, Viscidi R, Tsai YC, He L, Chen PJ, Boyd DA, Wu TC. Generation and characterization of DNA vaccines targeting the nucleocapsid protein of severe acute respiratory syndrome coronavirus. J Virol 2004; 78: 4638–4645.

Kliger Y, Levanon EY. Cloaked similarity between HIV-1 and SARS-CoV suggests an anti-SARS strategy. BMC Microbiol 2003; 3: 20.

Knowles SR, Phillips EJ, Dresser L, Matukas L. Common adverse events associated with the use of ribavirin for severe acute respiratory syndrome in Canada. Clin Infect Dis 2003; 37: 1139–1142.

Kong W-p, Xu L, Stadler K, Ulmer JB, Abrignani S, Rappuoli R, Nabel GJ. Modulation of the immune response to the severe acute respiratory syndrome spike glycoprotein by gene-based and inactivated virus immunization. J Virol 2005; 79: 13915–13923.

Krug RM, Yuan W, Noah DL, Latham AG. Intracellular warfare between human influenza viruses and human cells: the roles of the viral NS1 protein. Virology 2003; 309: 181–189.

Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, Tong S, Urbani C, Comer JA, Lim W, Rollin PE, Dowell SF, Ling AE, Humphrey CD, Shieh WJ, Guarner J, Paddock CD, Rota P, Fields B, DeRisi J, Yang JY, Cox N, Hughes JM, LeDuc JW, Bellini WJ, Anderson LJ. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 2003; 348: 1953–1966.

Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L, Zhang B, Liu G, Wang Z, Chappell M, Liu Y, Zheng D, Leibbrandt A, Wada T, Slutsky AS, Liu D, Qin C, Jiang C, Penninger JM. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med 2005; 11: 875–879.

Kuiken T, Fouchier RA, Schutten M, Rimmelzwaan GF, van Amerongen G, van Riel D, Laman JD, de Jong T, van Doornum G, Lim W, Ling AE, Chan PK, Tam JS, Zambon MC, Gopal R, Drosten C, van der Werf S, Escriou N, Manuguerra JC, Stohr K, Peiris JS, Osterhaus AD. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. Lancet 2003; 362: 263–270.

Kuiken T, Leighton FA, Fouchier RA, LeDuc JW, Peiris JS, Schudel A, Stohr K, Osterhaus AD. Public health. Pathogen surveillance in animals. Science 2005; 309: 1680–1681.

Kwan AC, Chau TN, Tong WL, Tsang OT, Tso EY, Chiu MC, Yu WC, Lai TS. Severe acute respiratory syndrome-related diarrhea. J Gastroenterol Hepatol 2005a; 20: 606–610.

Kwan MY, Chan WM, Ko PW, Leung CW, Chiu MC. Severe acute respiratory syndrome can be mild in children. Pediatr Infect Dis J 2004; 23: 1172–1174.

Kwan YW, Leung CW, Chiu MC. Diarrhoea as the presenting sign in an adolescent suffering from severe acute respiratory syndrome. Eur J Pediatr 2005b; 164: 227–230.

Lai MM, Cavanagh D. The molecular biology of coronaviruses. Adv Virus Res 1997; 48: 1–100.

Lai MYY, Cheng PKC, Lim WWL. Survival of severe acute respiratory syndrome coronavirus. Clin Infect Dis 2005a; 41: e67–e71.

Lai PC, Wong CM, Hedley AJ, Lo SV, Leung PY, Kong J, Leung GM. Understanding the spatial clustering of severe acute respiratory syndrome (SARS) in Hong Kong. Environ Health Perspect 2004; 112: 1550–1556.

Lai ST. Treatment of severe acute respiratory syndrome. Eur J Clin Microbiol Infect Dis 2005; 24: 583–591.

Lai TS, Keung Ng T, Seto WH, Yam L, Law KI, Chan J. Low prevalence of subclinical severe acute respiratory syndrome-associated coronavirus infection among hospital healthcare workers in Hong Kong. Scand J Infect Dis 2005b; 37: 500–503.

Lang ZW, Zhang LJ, Zhang SJ, Meng X, Li JQ, Song CZ, Sun L, Zhou YS, Dwyer DE. A clinicopathological study of three cases of severe acute respiratory syndrome (SARS). Pathology 2003; 35: 526–531.

Lau SKP, Woo PCY, Li KSM, Huang Y, Tsoi H-W, Wong BHL, Wong SSY, Leung S-Y, Chan K-H, Yuen K-Y. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. Proc Natl Acad Sci USA 2005; 102: 14040–14045.

Lau YL, Peiris JM. Pathogenesis of severe acute respiratory syndrome. Curr Opin Immunol 2005; 17: 404–410.

Law HK, Cheung CY, Ng HY, Sia SF, Chan YO, Luk W, Nicholls JM, Peiris JS, Lau YL. Chemokine upregulation in SARS coronavirus infected human monocyte derived dendritic cells. Blood 2005a; 106: 2366–2374.

Law PT, Wong CH, Au TC, Chuck CP, Kong SK, Chan PK, To KF, Lo AW, Chan JY, Suen YK, Chan HY, Fung KP, Waye MM, Sung JJ, Lo YM, Tsui SK. The 3a protein of severe acute respiratory syndrome-associated coronavirus induces apoptosis in Vero E6 cells. J Gen Virol 2005b; 86: 1921–1930.

Lee CH, Chen RF, Liu JW, Yeh WT, Chang JC, Liu PM, Eng HL, Lin MC, Yang KD. Altered p38 mitogen-activated protein kinase expression in different leukocytes with

increment of immunosuppressive mediators in patients with severe acute respiratory syndrome. J Immunol 2004; 172: 7841–7847.

Lee HK, Tso EY, Chau TN, Tsang OT, Choi KW, Lai TS. Asymptomatic severe acute respiratory syndrome-associated coronavirus infection. Emerg Infect Dis 2003a; 9: 1491–1492.

Lee N, Hui D, Wu A, Chan P, Cameron P, Joynt GM, Ahuja A, Yung MY, Leung CB, To KF, Lui SF, Szeto CC, Chung S, Sung JJ. A major outbreak of severe acute respiratory syndrome in Hong Kong. N Engl J Med 2003b; 348: 1986–1994.

Lee TW, Cherney MM, Huitema C, Liu J, James KE, Powers JC, Eltis LD, James MN. Crystal structures of the main peptidase from the SARS coronavirus inhibited by a substrate-like aza-peptide epoxide. J Mol Biol 2005; 353: 1137–1151.

Lee YS, Chen CH, Chao A, Chen ES, Wei ML, Chen LK, Yang KD, Lin MC, Wang YH, Liu JW, Eng HL, Chiang PC, Wu TS, Tsao KC, Huang CG, Tien YJ, Wang TH, Wang HS, Lee YS. Molecular signature of clinical severity in patients infected with severe acute respiratory syndrome coronavirus (SARS-CoV). BMC Genomics 2005b; 6: 132.

Leikina E, Delanoe-Ayari H, Melikov K, Cho M-S, Chen A, Waring AJ, Wang W, Xie Y, Loo JA, Lehrer RI, Chernomordik LV. Carbohydrate-binding molecules inhibit viral fusion and entry by crosslinking membrane glycoproteins. Nat Immunol 2005; 6: 995–1001.

Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, Delicat A, Paweska JT, Gonzalez J-P, Swanepoel R. Fruit bats as reservoirs of Ebola virus. Nature 2005; 438: 575–576.

Leung FC. Hong Kong SARS sequence. Science 2003; 301: 309–310.

Leung GM, Hedley AJ, Ho LM, Chau P, Wong IO, Thach TQ, Ghani AC, Donnelly CA, Fraser C, Riley S, Ferguson NM, Anderson RM, Tsang T, Leung PY, Wong V, Chan JC, Tsui E, Lo SV, Lam TH. The epidemiology of severe acute respiratory syndrome in the 2003 Hong Kong epidemic: an analysis of all 1755 patients. Ann Intern Med 2004; 141: 662–673.

Leung TF, Wong GW, Hon KL, Fok TF. Severe acute respiratory syndrome (SARS) in children: epidemiology, presentation and management. Paediatr Respir Rev 2003a; 4: 334–339.

Leung WK, To KF, Chan PK, Chan HL, Wu AK, Lee N, Yuen KY, Sung JJ. Enteric involvement of severe acute respiratory syndrome-associated coronavirus infection. Gastroenterology 2003b; 125: 1011–1017.

Li B-j, Tang Q, Cheng D, Qin C, Xie FY, Wei Q, Xu J, Liu Y, Zheng B-j, Woodle MC, Zhong N, Lu PY. Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in rhesus macaque. Nat Med 2005a; 11: 944–951.

Li F, Li W, Farzan M, Harrison SC. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science 2005b; 309: 1864–1868.

Li W, Greenough TC, Moore MJ, Vasilieva N, Somasundaran M, Sullivan JL, Farzan M, Choe H. Efficient replication of severe acute respiratory syndrome coronavirus in mouse cells is limited by murine angiotensin-converting enzyme 2. J Virol 2004a; 78: 11429–11433.

Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H, Farzan M. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 2003; 426: 450–454.

Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, McEachern J, Field H, Daszak P, Eaton BT, Zhang S, Wang L-F. Bats are natural reservoirs of SARS-like coronaviruses. Science 2005; 310: 676–679.

Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, McEachern J, Field H, Daszak P, Eaton BT, Zhang S, Wang L-F. Bats are natural reservoirs of SARS-like coronaviruses. Science 2005d; 310: 676–679.

Li W, Zhang C, Sui J, Kuhn JH, Moore MJ, Luo S, Wong SK, Huang IC, Xu K, Vasilieva N, Murakami A, He Y, Marasco WA, Guan Y, Choe H, Farzan M. Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2. Embo J 2005e; 24: 1634–1643.

Li Y, Duan S, Yu IT, Wong TW. Multi-zone modeling of probable SARS virus transmission by airflow between flats in Block E, Amoy Gardens. Indoor Air 2005f; 15: 96–111.

Li Y, Huang X, Yu IT, Wong TW, Qian H. Role of air distribution in SARS transmission during the largest nosocomial outbreak in Hong Kong. Indoor Air 2005g; 15: 83–95.

Li Y, Luo C, Li W, Xu Z, Zeng C, Bi S, Yu J, Wu J, Yang H. Structure-based preliminary analysis of immunity and virulence of SARS coronavirus. Viral Immunol 2004b; 17: 528–534.

Liang G, Chen Q, Xu J, Liu Y, Lim W, Peiris JS, Anderson LJ, Ruan L, Li H, Kan B, Di B, Cheng P, Chan KH, Erdman DD, Gu S, Yan X, Liang W, Zhou D, Haynes L, Duan S, Zhang X, Zheng H, Gao Y, Tong S, Li D, Fang L, Qin P, Xu W. Laboratory diagnosis of four recent sporadic cases of community-acquired SARS, Guangdong Province, China. Emerg Infect Dis 2004; 10: 1774–1781.

Liao CM, Chang CF, Liang HM. A probabilistic transmission dynamic model to assess indoor airborne infection risks. Risk Anal 2005a; 25: 1097–1107.

Liao QJ, Ye LB, Timani KA, Zeng YC, She YL, Ye L, Wu ZH. Activation of NF-kappaB by the full-length nucleocapsid protein of the SARS coronavirus. Acta Biochim Biophys Sin (Shanghai) 2005b; 37: 607–612.

Lin CF, Lei HY, Shiau AL, Liu CC, Liu HS, Yeh TM, Chen SH, Lin YS. Antibodies from dengue patient sera cross-react with endothelial cells and induce damage. J Med Virol 2003a; 69: 82–90.

Lin M, Tseng HK, Trejaut JA, Lee HL, Loo JH, Chu CC, Chen PJ, Su YW, Lim KH, Tsai ZU, Lin RY, Lin RS, Huang CH. Association of HLA Class I with severe acute respiratory syndrome coronavirus infection. BMC Med Genet 2003b; 4: 9.

Lin S, Lee CK, Lee SY, Kao CL, Lin CW, Wang AB, Hsu SM, Huang LS. Surface ultrastructure of SARS coronavirus revealed by atomic force microscopy. Cell Microbiol 2005a; 7: 1763–1770.

Lin YS, Lin CF, Fang YT, Kuo YM, Liao PC, Yeh TM, Hwa KY, Shieh CC, Yen JH, Wang HJ, Su IJ, Lei HY. Antibody to severe acute respiratory syndrome (SARS)-associated coronavirus spike protein domain 2 cross-reacts with lung epithelial cells and causes cytotoxicity. Clin Exp Immunol 2005b; 141: 500–508.

Liu S, Xiao G, Chen Y, He Y, Niu J, Escalante CR, Xiong H, Farmar J, Debnath AK, Tien P, Jiang S. Interaction between heptad repeat 1 and 2 regions in spike protein of SARS-associated coronavirus: implications for virus fusogenic mechanism and identification of fusion inhibitors. Lancet 2004; 363: 938–947.

Liu W. Molecular epidemiology of SARS-associated coronavirus, Beijing. Emerg Infect Dis 2005; 11: 1420–1424.

Lloyd-Smith JO, Galvani AP, Getz WM. Curtailing transmission of severe acute respiratory syndrome within a community and its hospital. Proc Biol Sci 2003; 270: 1979–1989.

Lloyd-Smith JO, Schreiber SJ, Kopp PE, Getz WM. Superspreading and the effect of individual variation on disease emergence. Nature 2005; 438: 355–359.

Long WH, Xiao HS, Gu XM, Zhang QH, Yang HJ, Zhao GP, Liu JH. A universal microarray for detection of SARS coronavirus. J Virol Methods 2004; 121: 57–63.

Loon SC, Teoh SC, Oon LL, Se-Thoe SY, Ling AE, Leo YS, Leong HN. The severe acute respiratory syndrome coronavirus in tears. Br J Ophthalmol 2004; 88: 861–863.

Magiorkinis G, Magiorkinis E, Paraskevis D, Vandamme AM, Van Ranst M, Moulton V, Hatzakis A. Phylogenetic analysis of the full-length SARS-CoV sequences: evidence for phylogenetic discordance in three genomic regions. J Med Virol 2004; 74: 369–372.

Mahony JB, Richardson S. Molecular diagnosis of severe acute respiratory syndrome: the state of the art. J Mol Diagn 2005; 7: 551–559.

Marra MA, Jones SJ, Astell CR, Holt RA, Brooks-Wilson A, Butterfield YS, Khattra J, Asano JK, Barber SA, Chan SY, Cloutier A, Coughlin SM, Freeman D, Girn N, Griffith OL, Leach SR, Mayo M, McDonald H, Montgomery SB, Pandoh PK, Petrescu AS, Robertson AG, Schein JE, Siddiqui A, Smailus DE, Stott JM, Yang GS, Plummer F, Andonov A, Artsob H, Bastien N, Bernard K, Booth TF, Bowness D, Czub M, Drebot M, Fernando L, Flick R, Garbutt M, Gray M, Grolla A, Jones S, Feldmann H, Meyers A, Kabani A, Li Y, Normand S, Stroher U, Tipples GA, Tyler S, Vogrig R, Ward D, Watson B, Brunham RC, Krajden M, Petric M, Skowronski DM, Upton C, Roper RL. The genome sequence of the SARS-associated coronavirus. Science 2003; 300: 1399–1404.

Martina BE, Haagmans BL, Kuiken T, Fouchier RA, Rimmelzwaan GF, Van Amerongen G, Peiris JS, Lim W, Osterhaus AD. Virology: SARS virus infection of cats and ferrets. Nature 2003; 425: 915.

Martina E, Stiefl N, Degel B, Schulz F, Breuning A, Schiller M, Vicik R, Baumann K, Ziebuhr J, Schirmeister T. Screening of electrophilic compounds yields an aziridinyl peptide as new active-site directed SARS-CoV main protease inhibitor. Bioorg Med Chem Lett 2005; 15: 5365–5369.

Marzi A, Gramberg T, Simmons G, Moller P, Rennekamp AJ, Krumbiegel M, Geier M, Eisemann J, Turza N, Saunier B, Steinkasserer A, Becker S, Bates P, Hofmann H, Pohlmann S. DC-SIGN and DC-SIGNR interact with the glycoprotein of Marburg virus and the S protein of severe acute respiratory syndrome coronavirus. J Virol 2004; 78: 12090–12095.

Matsuyama S, Ujike M, Morikawa S, Tashiro M, Taguchi F. Protease-mediated enhancement of severe acute respiratory syndrome coronavirus infection. Proc Natl Acad Sci USA. 2005; 102: 12543–12547.

Mazzulli T, Farcas GA, Poutanen SM, Willey BM, Low DE, Butany J, Asa SL, Kain KC. Severe acute respiratory syndrome-associated coronavirus in lung tissue. Emerg Infect Dis 2004; 10: 20–24.

Meng B, Wang J, Liu J, Wu J, Zhong E. Understanding the spatial diffusion process of severe acute respiratory syndrome in Beijing. Public Health 2005; 119: 1080–1087.

Morgenstern B, Michaelis M, Baer PC, Doerr HW, Cinatl Jr. J. Ribavirin and interferon-beta synergistically inhibit SARS-associated coronavirus replication in animal and human cell lines. Biochem Biophys Res Commun 2005; 326: 905–908.

Mortola E, Roy P. Efficient assembly and release of SARS coronavirus-like particles by a heterologous expression system. FEBS Lett 2004; 576: 174–178.
Ng EK, Ng PC, Hon KL, Cheng WT, Hung EC, Chan KC, Chiu RW, Li AM, Poon LL, Hui DS, Tam JS, Fok TF, Lo YM. Serial analysis of the plasma concentration of SARS coronavirus RNA in pediatric patients with severe acute respiratory syndrome. Clin Chem 2003; 49: 2085–2088.
Ng LF, Wong M, Koh S, Ooi EE, Tang KF, Leong HN, Ling AE, Agathe LV, Tan J, Liu ET, Ren EC, Ng LC, Hibberd ML. Detection of severe acute respiratory syndrome coronavirus in blood of infected patients. J Clin Microbiol 2004; 42: 347–350.
Ni L, Zhu J, Zhang J, Yan M, Gao GF, Tien P. Design of recombinant protein-based SARS-CoV entry inhibitors targeting the heptad-repeat regions of the spike protein S2 domain. Biochem Biophys Res Commun 2005; 330: 39–45.
Nicholls J, Peiris M. Good ACE, bad ACE do battle in lung injury, SARS. Nat Med 2005; 11: 821–822.
Nicholls JM, Poon LL, Lee KC, Ng WF, Lai ST, Leung CY, Chu CM, Hui PK, Mak KL, Lim W, Yan KW, Chan KH, Tsang NC, Guan Y, Yuen KY, Peiris JS. Lung pathology of fatal severe acute respiratory syndrome. Lancet 2003; 361: 1773–1778.
Normile D. Infectious diseases. Viral DNA match spurs China's civet roundup. Science 2004; 303: 292.
Normile D. Virology: researchers tie deadly SARS virus to bats. Science 2005; 309: 2154–2155.
Normile D, Enserink M. SARS in China. Tracking the roots of a killer. Science 2003; 301: 297–299.
Offit PA. The Cutter incident, 50 years later. N Engl J Med 2005; 352: 1411–1412.
Olsen SJ, Chang HL, Cheung TY, Tang AF, Fisk TL, Ooi SP, Kuo HW, Jiang DD, Chen KT, Lando J, Hsu KH, Chen TJ, Dowell SF. Transmission of the severe acute respiratory syndrome on aircraft. N Engl J Med 2003; 349: 2416–2422.
Ong KC, Ng AW, Lee LS, Kaw G, Kwek SK, Leow MK, Earnest A. 1-year pulmonary function and health status in survivors of severe acute respiratory syndrome. Chest 2005; 128: 1393–1400.
Pager CT, Dutch RE. Cathepsin L is involved in proteolytic processing of the Hendra virus fusion protein. J Virol 2005; 79: 12714–12720.
Pariente N, Sierra S, Lowenstein PR, Domingo E. Efficient virus extinction by combinations of a mutagen and antiviral inhibitors. J Virol 2001; 75: 9723–9730.
Parker WB. Metabolism and antiviral activity of ribavirin. Virus Res 2005; 107: 165–171.
Peiris JM. Joseph Malik Peiris—on the trail of pneumonia in Hong Kong interviewed by Pam Das. Lancet Infect Dis 2003; 3: 309–311.
Peiris JS, Chu CM, Cheng VC, Chan KS, Hung IF, Poon LL, Law KI, Tang BS, Hon TY, Chan CS, Chan KH, Ng JS, Zheng BJ, Ng WL, Lai RW, Guan Y, Yuen KY. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet 2003a; 361: 1767–1772.
Peiris JS, Lai ST, Poon LL, Guan Y, Yam LY, Lim W, Nicholls J, Yee WK, Yan WW, Cheung MT, Cheng VC, Chan KH, Tsang DN, Yung RW, Ng TK, Yuen KY. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 2003b; 361: 1319–1325.
Peiris JS, Yuen KY, Osterhaus AD, Stohr K. The severe acute respiratory syndrome. N Engl J Med 2003c; 349: 2431–2441.

Petit CM, Melancon JM, Chouljenko VN, Colgrove R, Farzan M, Knipe DM, Kousoulas KG. Genetic analysis of the SARS-coronavirus spike glycoprotein functional domains involved in cell-surface expression and cell-to-cell fusion. Virology 2005; 341: 215–230.

Poon LL, Chan KH, Wong OK, Yam WC, Yuen KY, Guan Y, Lo YM, Peiris JS. Early diagnosis of SARS coronavirus infection by real time RT-PCR. J Clin Virol 2003; 28: 233–238.

Poon LL, Chu DK, Chan KH, Wong OK, Ellis TM, Leung YH, Lau SK, Woo PC, Suen KY, Yuen KY, Guan Y, Peiris JS. Identification of a novel coronavirus in bats. J Virol 2005; 79: 2001–2009.

Poon LL, Guan Y, Nicholls JM, Yuen KY, Peiris JS. The aetiology, origins, and diagnosis of severe acute respiratory syndrome. Lancet Infect Dis 2004a; 4: 663–671.

Poon LL, Wong BW, Chan KH, Leung CS, Yuen KY, Guan Y, Peiris JS. A one step quantitative RT-PCR for detection of SARS coronavirus with an internal control for PCR inhibitors. J Clin Virol 2004b; 30: 214–217.

Poon TC, Chan KC, Ng PC, Chiu RW, Ang IL, Tong YK, Ng EK, Cheng FW, Li AM, Hon EK, Fok TF, Lo YM. Serial analysis of plasma proteomic signatures in pediatric patients with severe acute respiratory syndrome and correlation with viral load. Clin Chem 2004c; 50: 1452–1455.

Prentice E, McAuliffe J, Lu X, Subbarao K, Denison MR. Identification and characterization of severe acute respiratory syndrome coronavirus replicase proteins. J Virol 2004; 78: 9977–9986.

Qin L, Xiong B, Luo C, Guo ZM, Hao P, Su J, Nan P, Feng Y, Shi YX, Yu XJ, Luo XM, Chen KX, Shen X, Shen JH, Zou JP, Zhao GP, Shi TL, He WZ, Zhong Y, Jiang HL, Li YX. Identification of probable genomic packaging signal sequence from SARS-CoV genome by bioinformatics analysis. Acta Pharmacol Sin 2003; 24: 489–496.

Reghunathan R, Jayapal M, Hsu LY, Chng HH, Tai D, Leung BP, Melendez AJ. Expression profile of immune response genes in patients with severe acute respiratory syndrome. BMC Immunol 2005; 6: 2.

Reilley B, Van Herp M, Sermand D, Dentico N. SARS and Carlo Urbani. N Engl J Med 2003; 348: 1951–1952.

Riley S, Fraser C, Donnelly CA, Ghani AC, Abu-Raddad LJ, Hedley AJ, Leung GM, Ho LM, Lam TH, Thach TQ, Chau P, Chan KP, Lo SV, Leung PY, Tsang T, Ho W, Lee KH, Lau EM, Ferguson NM, Anderson RM. Transmission dynamics of the etiological agent of SARS in Hong Kong: impact of public health interventions. Science 2003; 300: 1961–1966.

Roberts A, Vogel L, Guarner J, Hayes N, Murphy B, Zaki S, Subbarao K. Severe acute respiratory syndrome coronavirus infection of golden Syrian hamsters. J Virol 2005; 79: 503–511.

Rocha EP, Danchin A. Base composition bias might result from competition for metabolic resources. Trends Genet 2002; 18: 291–294.

Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, Icenogle JP, Penaranda S, Bankamp B, Maher K, Chen MH, Tong S, Tamin A, Lowe L, Frace M, DeRisi JL, Chen Q, Wang D, Erdman DD, Peret TC, Burns C, Ksiazek TG, Rollin PE, Sanchez A, Liffick S, Holloway B, Limor J, McCaustland K, Olsen-Rasmussen M, Fouchier R, Gunther S, Osterhaus AD, Drosten C, Pallansch MA, Anderson LJ, Bellini WJ. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 2003; 300: 1394–1399.

Roy CJ, Milton DK. Airborne transmission of communicable infection—the elusive pathway. N Engl J Med 2004; 350: 1710–1712.

Ruan YJ, Wei CL, Ee AL, Vega VB, Thoreau H, Su ST, Chia JM, Ng P, Chiu KP, Lim L, Zhang T, Peng CK, Lin EO, Lee NM, Yee SL, Ng LF, Chee RE, Stanton LW, Long PM, Liu ET. Comparative full-length genome sequence analysis of 14 SARS coronavirus isolates and common mutations associated with putative origins of infection. Lancet 2003; 361: 1779–1785.

Sachdeva G, Kumar K, Jain P, Ramachandran S. SPAAN: a software program for prediction of adhesins and adhesin-like proteins using neural networks. Bioinformatics 2005; 21: 483–491.

Saikatendu KS, Joseph JS, Subramanian V, Clayton T, Griffith M, Moy K, Velasquez J, Neuman BW, Buchmeier MJ, Stevens RC, Kuhn P. Structural basis of severe acute respiratory syndrome coronavirus ADP-ribose-1''-phosphate dephosphorylation by a conserved domain of nsP3. Structure (Camb) 2005; 13: 1665–1675.

Samuel CE. Antiviral actions of interferons. Clin Microbiol Rev 2001; 14: 778–809.

Santoro MG, Rossi A, Amici C. New EMBO member's review: NF-{kappa}B and virus infection: who controls whom. EMBO J 2003; 22: 2552–2560.

Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases? Lancet Infect Dis 2003; 3: 722–727.

Schmidt M, Brixner V, Ruster B, Hourfar MK, Drosten C, Preiser W, Seifried E, Roth WK. NAT screening of blood donors for severe acute respiratory syndrome coronavirus can potentially prevent transfusion associated transmissions. Transfusion 2004; 44: 470–475.

Seto WH, Tsang D, Yung RW, Ching TY, Ng TK, Ho M, Ho LM, Peiris JS. Effectiveness of precautions against droplets and contact in prevention of nosocomial transmission of severe acute respiratory syndrome (SARS). Lancet 2003; 361: 1519–1520.

Shek CC, Ng PC, Fung GP, Cheng FW, Chan PK, Peiris MJ, Lee KH, Wong SF, Cheung HM, Li AM, Hon EK, Yeung CK, Chow CB, Tam JS, Chiu MC, Fok TF. Infants born to mothers with severe acute respiratory syndrome. Pediatrics 2003; 112: e254.

Shen X, Xue JH, Yu CY, Luo HB, Qin L, Yu XJ, Chen J, Chen LL, Xiong B, Yue LD, Cai JH, Shen JH, Luo XM, Chen KX, Shi TL, Li YX, Hu GX, Jiang HL. Small envelope protein E of SARS: cloning, expression, purification, CD determination, and bioinformatics analysis. Acta Pharmacol Sin 2003; 24: 505–511.

Shen Z, Ning F, Zhou W, He X, Lin C, Chin DP, Zhu Z, Schuchat A. Superspreading SARS events, Beijing, 2003. Emerg Infect Dis 2004; 10: 256–260.

Shi Y, Yang de H, Xiong J, Jia J, Huang B, Jin YX. Inhibition of genes expression of SARS coronavirus by synthetic small interfering RNAs. Cell Res 2005; 15: 193–200.

Shieh WJ, Hsiao CH, Paddock CD, Guarner J, Goldsmith CS, Tatti K, Packard M, Mueller L, Wu MZ, Rollin P, Su IJ, Zaki SR. Immunohistochemical, *in situ* hybridization, and ultrastructural localization of SARS-associated coronavirus in lung of a fatal case of severe acute respiratory syndrome in Taiwan. Hum Pathol 2005; 36: 303–309.

Simmons G, Gosalia DN, Rennekamp AJ, Reeves JD, Diamond SL, Bates P. Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. Proc Natl Acad Sci USA 2005; 102: 11876–11881.

Sims AC, Baric RS, Yount B, Burkett SE, Collins PL, Pickles RJ. Severe acute respiratory syndrome coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs. J Virol 2005; 79: 15511–15524.

Snijder EJ, Bredenbeek PJ, Dobbe JC, Thiel V, Ziebuhr J, Poon LL, Guan Y, Rozanov M, Spaan WJ, Gorbalenya AE. Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. J Mol Biol 2003; 331: 991–1004.

Song E, Zhu P, Lee S-K, Chowdhury D, Kussman S, Dykxhoorn DM, Feng Y, Palliser D, Weiner DB, Shankar P, Marasco WA, Lieberman J. Antibody mediated *in vivo* delivery of small interfering RNAs via cell-surface receptors. Nat Biotech 2005a; 23: 709–717.

Song HD, Tu CC, Zhang GW, Wang SY, Zheng K, Lei LC, Chen QX, Gao YW, Zhou HQ, Xiang H, Zheng HJ, Chern SW, Cheng F, Pan CM, Xuan H, Chen SJ, Luo HM, Zhou DH, Liu YF, He JF, Qin PZ, Li LH, Ren YQ, Liang WJ, Yu YD, Anderson L, Wang M, Xu RH, Wu XW, Zheng HY, Chen JD, Liang G, Gao Y, Liao M, Fang L, Jiang LY, Li H, Chen F, Di B, He LJ, Lin JY, Tong S, Kong X, Du L, Hao P, Tang H, Bernini A, Yu XJ, Spiga O, Guo ZM, Pan HY, He WZ, Manuguerra JC, Fontanet A, Danchin A, Niccolai N, Li YX, Wu CI, Zhao GP. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci USA 2005b; 102: 2430–2435.

Spiegel M, Pichlmair A, Martinez-Sobrido L, Cros J, Garcia-Sastre A, Haller O, Weber F. Inhibition of beta interferon induction by severe acute respiratory syndrome coronavirus suggests a two-step model for activation of interferon regulatory factor 3. J Virol 2005; 79: 2079–2086.

Spiegel M, Pichlmair A, Muhlberger E, Haller O, Weber F. The antiviral effect of interferon-beta against SARS-coronavirus is not mediated by MxA protein. J Clin Virol 2004; 30: 211–213.

Spruth M, Kistner O, Savidis-Dacho H, Hitter E, Crowe B, Gerencer M, Bruhl P, Grillberger L, Reiter M, Tauer C, Mundt W, Barrett PN. A double-inactivated whole virus candidate SARS coronavirus vaccine stimulates neutralising and protective antibody responses. Vaccine 2005; 24: 652–661.

Stadler K, Masignani V, Eickmann M, Becker S, Abrignani S, Klenk HD, Rappuoli R. SARS—beginning to understand a new virus. Nat Rev Microbiol 2003; 1: 209–218.

Stafford N. Profile: Klaus Stohr: preparing for the next influenza pandemic. Lancet 2005; 365: 379.

Su MC, Chang CT, Chu CH, Tsai CH, Chang KY. An atypical RNA pseudoknot stimulator and an upstream attenuation signal for 1-ribosomal frameshifting of SARS coronavirus. Nucleic Acids Res 2005; 33: 4265–4275.

Sui J, Li W, Murakami A, Tamin A, Matthews LJ, Wong SK, Moore MJ, Tallarico AS, Olurinde M, Choe H, Anderson LJ, Bellini WJ, Farzan M, Marasco WA. Potent neutralization of severe acute respiratory syndrome (SARS) coronavirus by a human mAb to S1 protein that blocks receptor association. Proc Natl Acad Sci USA 2004; 101: 2536–2541.

Sui J, Li W, Roberts A, Matthews LJ, Murakami A, Vogel L, Wong SK, Subbarao K, Farzan M, Marasco WA. Evaluation of human monoclonal antibody 80R for immunoprophylaxis of severe acute respiratory syndrome by an animal study, epitope mapping, and analysis of spike variants. J Virol 2005; 79: 5900–5906.

Supekar VM, Bruckmann C, Ingallinella P, Bianchi E, Pessi A, Carfi A. Structure of a proteolytically resistant core from the severe acute respiratory syndrome coronavirus S2 fusion protein. Proc Natl Acad Sci USA 2004; 101: 17958–17963.

Surjit M, Kumar R, Mishra RN, Reddy MK, Chow VT, Lal SK. The severe acute respiratory syndrome coronavirus nucleocapsid protein is phosphorylated and localizes in the cytoplasm by 14-3-3-mediated translocation. J Virol 2005; 79: 11476–11486.

Sylvester-Hvid C, Nielsen M, Lamberth K, Roder G, Justesen S, Lundegaard C, Worning P, Thomadsen H, Lund O, Brunak S, Buus S. SARS CTL vaccine candidates; HLA supertype-, genome-wide scanning and biochemical validation. Tissue Antigens 2004; 63: 395–400.

Takasuka N, Fujii H, Takahashi Y, Kasai M, Morikawa S, Itamura S, Ishii K, Sakaguchi M, Ohnishi K, Ohshima M, Hashimoto S, Odagiri T, Tashiro M, Yoshikura H, Takemori T, Tsunetsugu-Yokota Y. A subcutaneously injected UV-inactivated SARS coronavirus vaccine elicits systemic humoral immunity in mice. Int Immunol 2004; 16: 1423–1430.

Talaat AM, Stemke-Hale K. Expression library immunization: a road map for discovery of vaccines against infectious diseases. Infect Immun 2005; 73: 7089–7098.

Tan YJ, Fielding BC, Goh PY, Shen S, Tan TH, Lim SG, Hong W. Overexpression of 7a, a protein specifically encoded by the severe acute respiratory syndrome coronavirus, induces apoptosis via a caspase-dependent pathway. J Virol 2004a; 78: 14043–14047.

Tan YJ, Teng E, Shen S, Tan TH, Goh PY, Fielding BC, Ooi EE, Tan HC, Lim SG, Hong W. A novel severe acute respiratory syndrome coronavirus protein, U274, is transported to the cell surface and undergoes endocytosis. J Virol 2004b; 78: 6723–6734.

Tang L, Zhu Q, Qin E, Yu M, Ding Z, Shi H, Cheng X, Wang C, Chang G, Zhu Q, Fang F, Chang H, Li S, Zhang X, Chen X, Yu J, Wang J, Chen Z. Inactivated SARS-CoV vaccine prepared from whole virus induces a high level of neutralizing antibodies in BALB/c mice. DNA Cell Biol 2004; 23: 391–394.

Tang NL, Chan PK, Wong CK, To KF, Wu AK, Sung YM, Hui DS, Sung JJ, Lam CW. Early enhanced expression of interferon-inducible protein-10 (CXCL-10) and other chemokines predicts adverse outcome in severe acute respiratory syndrome. Clin Chem 2005; 51: 2333–2340.

Tao P, Zhang J, Tang N, Zhang BQ, He TC, Huang AL. Potent and specific inhibition of SARS-CoV antigen expression by RNA interference. Chin Med J (Engl) 2005; 118: 714–719.

Taylor DR. Obstacles and advances in SARS vaccine development. Vaccine 2005; 24: 863–871.

Temperton NJ, Chan PK, Simmons G, Zambon MC, Tedder RS, Takeuchi Y, Weiss RA. Longitudinally profiling neutralizing antibody response to SARS coronavirus with pseudotypes. Emerg Infect Dis 2005; 11: 411–416.

ter Meulen J, Bakker AB, van den Brink EN, Weverling GJ, Martina BE, Haagmans BL, Kuiken T, de Kruif J, Preiser W, Spaan W, Gelderblom HR, Goudsmit J, Osterhaus AD. Human monoclonal antibody as prophylaxis for SARS coronavirus infection in ferrets. Lancet 2004; 363: 2139–2141.

Thiel V, Ivanov KA, Putics A, Hertzig T, Schelle B, Bayer S, Weissbrich B, Snijder EJ, Rabenau H, Doerr HW, Gorbalenya AE, Ziebuhr J. Mechanisms and enzymes involved in SARS coronavirus genome expression. J Gen Virol 2003; 84: 2305–2315.

Thomas HC, Torok ME, Forton DM, Taylor-Robinson SD. Possible mechanisms of action and reasons for failure of antiviral therapy in chronic hepatitis C. J Hepatol 1999; 31(Suppl 1): 152–159.

Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. J Biol Chem 2000; 275: 33238–33243.

To KF, Tong JH, Chan PK, Au FW, Chim SS, Chan KC, Cheung JL, Liu EY, Tse GM, Lo AW, Lo YM, Ng HK. Tissue and cellular tropism of the coronavirus associated with severe acute respiratory syndrome: an *in-situ* hybridization study of fatal cases. J Pathol 2004; 202: 157–163.

Tong T, Lai TS. The severe acute respiratory syndrome coronavirus in tears. Br J Ophthalmol 2005; 89: 392.

Tong TR. SARS infection control. Lancet 2003; 362: 76–77.

Tong TR. Airborne severe acute respiratory syndrome coronavirus and its implications. J Infect Dis 2005a; 191: 1401–1402.

Tong TR. SARS-CoV sampling from 3 portals. Emerg Infect Dis 2005b; 11: 167.

Tong TR, Lam BH, Ng TK, Lai ST, Tong MK, Chau TN. Conjunctiva-upper respiratory tract irrigation for early diagnosis of severe acute respiratory syndrome. J Clin Microbiol 2003; 41: 5352.

Tong TR, Liang C. Evidence of airborne transmission of SARS. N Engl J Med 2004; 351: 609–611.

Traggiai E, Becker S, Subbarao K, Kolesnikova L, Uematsu Y, Gismondo MR, Murphy BR, Rappuoli R, Lanzavecchia A. An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus. Nat Med 2004; 10: 871–875.

Tripet B, Howard MW, Jobling M, Holmes RK, Holmes KV, Hodges RS. Structural characterization of the SARS-coronavirus spike S fusion protein core. J Biol Chem 2004; 279: 20836–20849.

Tsang KW, Ho PL, Ooi GC, Yee WK, Wang T, Chan-Yeung M, Lam WK, Seto WH, Yam LY, Cheung TM, Wong PC, Lam B, Ip MS, Chan J, Yuen KY, Lai KN. A cluster of cases of severe acute respiratory syndrome in Hong Kong. N Engl J Med 2003a; 348: 1977–1985.

Tsang OT, Chau TN, Choi KW, Tso EY, Lim W, Chiu MC, Tong WL, Lee PO, Lam BH, Ng TK, Lai JY, Yu WC, Lai ST. Coronavirus-positive nasopharyngeal aspirate as predictor for severe acute respiratory syndrome mortality. Emerg Infect Dis 2003b; 9: 1381–1387.

Tse GM, To KF, Chan PK, Lo AW, Ng KC, Wu A, Lee N, Wong HC, Mak SM, Chan KF, Hui DS, Sung JJ, Ng HK. Pulmonary pathological features in coronavirus associated severe acute respiratory syndrome (SARS). J Clin Pathol 2004; 57: 260–265.

Tsui PT, Kwok ML, Yuen H, Lai ST. Severe acute respiratory syndrome: clinical outcome and prognostic correlates. Emerg Infect Dis 2003; 9: 1064–1069.

Tu C, Crameri G, Kong X, Chen J, Sun Y, Yu M, Xiang H, Xia X, Liu S, Ren T, Yu Y, Eaton BT, Xuan H, Wang LF. Antibodies to SARS coronavirus in civets. Emerg Infect Dis 2004; 10: 2244–2248.

van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RAM, Osterhaus ADME. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med 2001; 7: 719–724.

van Vonderen MG, Bos JC, Prins JM, Wertheim-van Dillen P, Speelman P. Ribavirin in the treatment of severe acute respiratory syndrome (SARS). Neth J Med 2003; 61: 238–241.

Vega VB, Ruan Y, Liu J, Lee WH, Wei CL, Se-Thoe SY, Tang KF, Zhang T, Kolatkar PR, Ooi EE, Ling AE, Stanton LW, Long PM, Liu ET. Mutational dynamics of the SARS coronavirus in cell culture and human populations isolated in 2003. BMC Infect Dis 2004; 4: 32.

Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, Seidah NG, Nichol ST. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J 2005; 2: 69.

von Grotthuss M, Wyrwicz LS, Rychlewski L. mRNA cap-1 methyltransferase in the SARS genome. Cell 2003; 113: 701–702.

Wang D, Lu J. Glycan arrays lead to the discovery of autoimmunogenic activity of SARS-CoV. Physiol Genomics 2004; 18: 245–248.

Wang D, Urisman A, Liu YT, Springer M, Ksiazek TG, Erdman DD, Mardis ER, Hickenbotham M, Magrini V, Eldred J, Latreille JP, Wilson RK, Ganem D, DeRisi JL. Viral discovery and sequence recovery using DNA microarrays. PLoS Biol 2003; 1: E2.

Wang JT, Sheng WH, Fang CT, Chen YC, Wang JL, Yu CJ, Chang SC, Yang PC. Clinical manifestations, laboratory findings, and treatment outcomes of SARS patients. Emerg Infect Dis 2004a; 10: 818–824.

Wang S, Browning KS, Miller WA. A viral sequence in the 3′-untranslated region mimics a 5′ cap in facilitating translation of uncapped mRNA. EMBO J 1997; 16: 4107–4116.

Wang XW, Li J, Guo T, Zhen B, Kong Q, Yi B, Li Z, Song N, Jin M, Xiao W, Zhu X, Gu C, Yin J, Wei W, Yao W, Liu C, Li J, Ou G, Wang M, Fang T, Wang G, Qiu Y, Wu H, Chao F, Li J. Concentration and detection of SARS coronavirus in sewage from Xiao Tang Shan Hospital and the 309th Hospital of the Chinese People's Liberation Army. Water Sci Technol 2005a; 52: 213–221.

Wang XW, Li JS, Guo TK, Zhen B, Kong QX, Yi B, Li Z, Song N, Jin M, Wu XM, Xiao WJ, Zhu XM, Gu CQ, Yin J, Wei W, Yao W, Liu C, Li JF, Ou GR, Wang MN, Fang TY, Wang GJ, Qiu YH, Wu HH, Chao FH, Li JW. Excretion and detection of SARS coronavirus and its nucleic acid from digestive system. World J Gastroenterol 2005b; 11: 4390–4395.

Wang YS, Sun SH, Shen H, Jiang LH, Zhang MX, Xiao DJ, Liu Y, Ma XL, Zhang Y, Guo NJ, Jia TH. Cross-reaction of SARS-CoV antigen with autoantibodies in autoimmune diseases. Cell Mol Immunol 2004b; 1: 304–307.

Wang Z, Ren L, Zhao X, Hung T, Meng A, Wang J, Chen YG. Inhibition of severe acute respiratory syndrome virus replication by small interfering RNAs in mammalian cells. J Virol 2004c; 78: 7523–7527.

Wang Z, Yuan Z, Matsumoto M, Hengge UR, Chang YF. Immune responses with DNA vaccines encoded different gene fragments of severe acute respiratory syndrome coronavirus in BALB/c mice. Biochem Biophys Res Commun 2005c; 327: 130–135.

Webby R, Hoffmann E, Webster R. Molecular constraints to interspecies transmission of viral pathogens. Nat Med 2004; 10: S77–S81.

Wentworth DE, Gillim-Ross L, Espina N, Bernard KA. Mice susceptible to SARS coronavirus. Emerg Infect Dis 2004; 10: 1293–1296.

Wong CK, Lam CW, Wu AK, Ip WK, Lee NL, Chan IH, Lit LC, Hui DS, Chan MH, Chung SS, Sung JJ. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. Clin Exp Immunol 2004a; 136: 95–103.

Wong RS, Wu A, To KF, Lee N, Lam CW, Wong CK, Chan PK, Ng MH, Yu LM, Hui DS, Tam JS, Cheng G, Sung JJ. Haematological manifestations in patients with severe acute respiratory syndrome: retrospective analysis. BMJ 2003a; 326: 1358–1362.

Wong SK, Li W, Moore MJ, Choe H, Farzan M. A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. J Biol Chem 2004b; 279: 3197–3201.

Wong VW, Dai D, Wu AK, Sung JJ. Treatment of severe acute respiratory syndrome with convalescent plasma. Hong Kong Med J 2003b; 9: 199–201.

Woo PC, Lau SK, Wong BH, Chan KH, Hui WT, Kwan GS, Peiris JS, Couch RB, Yuen KY. False-positive results in a recombinant severe acute respiratory syndrome-associated coronavirus (SARS-CoV) nucleocapsid enzyme-linked immunosorbent assay due to HCoV-OC43 and HCoV-229E rectified by Western blotting with recombinant SARS-CoV spike polypeptide. J Clin Microbiol 2004a; 42: 5885–5888.

Woo PC, Lau SK, Wong BH, Tsoi HW, Fung AM, Chan KH, Tam VK, Peiris JS, Yuen KY. Detection of specific antibodies to severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein for serodiagnosis of SARS coronavirus pneumonia. J Clin Microbiol 2004b; 42: 2306–2309.

Wu CJ, Huang HW, Liu CY, Hong CF, Chan YL. Inhibition of SARS-CoV replication by siRNA. Antiviral Res 2005a; 65: 45–48.

Wu CY, Jan JT, Ma SH, Kuo CJ, Juan HF, Cheng YS, Hsu HH, Huang HC, Wu D, Brik A, Liang FS, Liu RS, Fang JM, Chen ST, Liang PH, Wong CH. Small molecules targeting severe acute respiratory syndrome human coronavirus. Proc Natl Acad Sci USA 2004; 101: 10012–10017.

Wu D, Tu C, Xin C, Xuan H, Meng Q, Liu Y, Yu Y, Guan Y, Jiang Y, Yin X, Crameri G, Wang M, Li C, Liu S, Liao M, Feng L, Xiang H, Sun J, Chen J, Sun Y, Gu S, Liu N, Fu D, Eaton BT, Wang LF, Kong X. Civets are equally susceptible to experimental infection by two different severe acute respiratory syndrome coronavirus isolates. J Virol 2005b; 79: 2620–2625.

Xu D, Zhang Z, Chu F, Li Y, Jin L, Zhang L, Gao GF, Wang FS. Genetic variation of SARS coronavirus in Beijing Hospital. Emerg Infect Dis 2004a; 10: 789–794.

Xu D, Zhang Z, Wang FS. SARS-associated coronavirus quasispecies in individual patients. N Engl J Med 2004b; 350: 1366–1367.

Xu J, Qi L, Chi X, Yang J, Wei X, Gong E, Peh S, Gu J. Orchitis: a complication of severe acute respiratory syndrome (SARS). Biol Reprod 2006; 74: 410–416.

Xu RH, He JF, Evans MR, Peng GW, Field HE, Yu DW, Lee CK, Luo HM, Lin WS, Lin P, Li LH, Liang WJ, Lin JY, Schnur A. Epidemiologic clues to SARS origin in China. Emerg Infect Dis 2004c; 10: 1030–1037.

Xu Y, Liu Y, Lou Z, Qin L, Li X, Bai Z, Pang H, Tien P, Gao GF, Rao Z. Structural basis for coronavirus-mediated membrane fusion. Crystal structure of mouse hepatitis virus spike protein fusion core. J Biol Chem 2004d; 279: 30514–30522.

Xu Y, Lou Z, Liu Y, Pang H, Tien P, Gao GF, Rao Z. Crystal structure of severe acute respiratory syndrome coronavirus spike protein fusion core. J Biol Chem 2004e; 279: 49414–49419.

Yamamoto N, Yang R, Yoshinaka Y, Amari S, Nakano T, Cinatl J, Rabenau H, Doerr HW, Hunsmann G, Otaka A, Tamamura H, Fujii N, Yamamoto N. HIV protease inhibitor nelfinavir inhibits replication of SARS-associated coronavirus. Biochem Biophys Res Commun 2004; 318: 719–725.

Yamate M, Yamashita M, Goto T, Tsuji S, Li YG, Warachit J, Yunoki M, Ikuta K. Establishment of Vero E6 cell clones persistently infected with severe acute respiratory syndrome coronavirus. Microbes Infect 2005; 7: 1530–1540.

Yang H, Xie W, Xue X, Yang K, Ma J, Liang W, Zhao Q, Zhou Z, Pei D, Ziebuhr J, Hilgenfeld R, Yuen KY, Wong L, Gao G, Chen S, Chen Z, Ma D, Bartlam M, Rao Z. Design of wide-spectrum inhibitors targeting coronavirus main proteases. PLoS Biol 2005a; 3: e324.

Yang Y, Xiong Z, Zhang S, Yan Y, Nguyen J, Ng B, Lu H, Brendese J, Yang F, Wang H, Yang XF. Bcl-xL inhibits T cell apotosis induced by expression of SARS coronavirus E protein in the absence of growth factors. Biochem J 2005; 392: 135–143.

Yang ZY, Huang Y, Ganesh L, Leung K, Kong WP, Schwartz O, Subbarao K, Nabel GJ. pH-dependent entry of severe acute respiratory syndrome coronavirus is mediated by the spike glycoprotein and enhanced by dendritic cell transfer through DC-SIGN. J Virol 2004a; 78: 5642–5650.

Yang ZY, Kong WP, Huang Y, Roberts A, Murphy BR, Subbarao K, Nabel GJ. A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. Nature 2004b; 428: 561–564.

Yang ZY, Werner HC, Kong WP, Leung K, Traggiai E, Lanzavecchia A, Nabel GJ. Evasion of antibody neutralization in emerging severe acute respiratory syndrome coronaviruses. Proc Natl Acad Sci USA 2005c; 102: 797–801.

Yeh KM, Chiueh TS, Siu LK, Lin JC, Chan PK, Peng MY, Wan HL, Chen JH, Hu BS, Perng CL, Lu JJ, Chang FY. Experience of using convalescent plasma for severe acute respiratory syndrome among healthcare workers in a Taiwan hospital. J Antimicrob Chemother 2005; 56: 919–922.

Yeh SH, Wang HY, Tsai CY, Kao CL, Yang JY, Liu HW, Su IJ, Tsai SF, Chen DS, Chen PJ. Characterization of severe acute respiratory syndrome coronavirus genomes in Taiwan: molecular epidemiology and genome evolution. Proc Natl Acad Sci USA 2004; 101: 2542–2547.

Yi CE, Ba L, Zhang L, Ho DD, Chen Z. Single amino acid substitutions in the severe acute respiratory syndrome coronavirus spike glycoprotein determine viral entry and immunogenicity of a major neutralizing domain. J Virol 2005; 79: 11638–11646.

Yip SP, To SST, Leung PHM, Cheung TS, Cheng PKC, Lim WWL. Use of dual TaqMan probes to increase the sensitivity of 1-step quantitative reverse transcription-PCR: application to the detection of SARS coronavirus. Clin Chem 2005; 51: 1885–1888.

Yount B, Curtis KM, Fritz EA, Hensley LE, Jahrling PB, Prentice E, Denison MR, Geisbert TW, Baric RS. Reverse genetics with a full-length infectious cDNA of severe acute respiratory syndrome coronavirus. Proc Natl Acad Sci USA 2003; 100: 12995–13000.

Yount B, Denison MR, Weiss SR, Baric RS. Systematic assembly of a full-length infectious cDNA of mouse hepatitis virus strain A59. J Virol 2002; 76: 11065–11078.

Yount B, Roberts RS, Sims AC, Deming D, Frieman MB, Sparks J, Denison MR, Davis N, Baric RS. Severe acute respiratory syndrome coronavirus group-specific open reading frames encode nonessential functions for replication in cell cultures and mice. J Virol 2005; 79: 14909–14922.

Yu CJ, Chen YC, Hsiao CH, Kuo TC, Chang SC, Lu CY, Wei WC, Lee CH, Huang LM, Chang MF, Ho HN, Lee FJ. Identification of a novel protein 3a from severe acute respiratory syndrome coronavirus. FEBS Lett 2004a; 565: 111–116.

Yu IT, Li Y, Wong TW, Tam W, Chan AT, Lee JH, Leung DY, Ho T. Evidence of airborne transmission of the severe acute respiratory syndrome virus. N Engl J Med 2004b; 350: 1731–1739.

Yu IT, Sung JJ. The epidemiology of the outbreak of severe acute respiratory syndrome (SARS) in Hong Kong—what we do know and what we don't. Epidemiol Infect 2004; 132: 781–786.

Yu IT, Wong TW, Chiu YL, Lee N, Li Y. Temporal-spatial analysis of severe acute respiratory syndrome among hospital inpatients. Clin Infect Dis 2005; 40: 1237–1243.

Yuan XD, Shan Y, Zhao Z, Chen J, Cong Y. G0/G1 arrest and apoptosis induced by SARS-CoV 3b protein in transfected cells. Virol J 2005; 2: 66.

Zeng F, Chow KY, Hon CC, Law KM, Yip CW, Chan KH, Peiris JS, Leung FC. Characterization of humoral responses in mice immunized with plasmid DNAs encoding SARS-CoV spike gene fragments. Biochem Biophys Res Commun 2004a; 315: 1134–1139.

Zeng R, Ruan HQ, Jiang XS, Zhou H, Shi L, Zhang L, Sheng QH, Tu Q, Xia QC, Wu JR. Proteomic analysis of SARS associated coronavirus using two-dimensional liquid chromatography mass spectrometry and one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by mass spectrometric analysis. J Proteome Res 2004b; 3: 549–555.

Zhai Y, Sun F, Li X, Pang H, Xu X, Bartlam M, Rao Z. Insights into SARS-CoV transcription and replication from the structure of the nsp7–nsp8 hexadecamer. Nat Struct Mol Biol 2005; 12: 980–986.

Zhang DM, Wang GL, Lu JH. Severe acute respiratory syndrome: vaccine on the way. Chin Med J (Engl) 2005a; 118: 1468–1476.

Zhang H, Wang G, Li J, Nie Y, Shi X, Lian G, Wang W, Yin X, Zhao Y, Qu X, Ding M, Deng H. Identification of an antigenic determinant on the S2 domain of the severe acute respiratory syndrome coronavirus spike glycoprotein capable of inducing neutralizing antibodies. J Virol 2004; 78: 6938–6945.

Zhang H, Zhou G, Zhi L, Yang H, Zhai Y, Dong X, Zhang X, Gao X, Zhu Y, He F. Association between mannose-binding lectin gene polymorphisms and susceptibility to severe acute respiratory syndrome coronavirus infection. J Infect Dis 2005b; 192: 1355–1361.

Zhang L, Zhang F, Yu W, He T, Yu J, Yi CE, Ba L, Li W, Farzan M, Chen Z, Yuen KY, Ho D. Antibody responses against SARS coronavirus are correlated with disease outcome of infected individuals. J Med Virol 2005c; 78: 1–8.

Zhang MY, Choudhry V, Xiao X, Dimitrov DS. Human monoclonal antibodies to the S glycoprotein and related proteins as potential therapeutics for SARS. Curr Opin Mol Ther 2005d; 7: 151–156.

Zhang R, Guo Z, Lu J, Meng J, Zhou C, Zhan X, Huang B, Yu X, Huang M, Pan X, Ling W, Chen X, Wan Z, Zheng H, Yan X, Wang Y, Ran Y, Liu X, Ma J, Wang C, Zhang B. Inhibiting severe acute respiratory syndrome-associated coronavirus by small interfering RNA. Chin Med J (Engl) 2003; 116: 1262–1264.

Zhang XW, Yap YL. Old drugs as lead compounds for a new disease? Binding analysis of SARS coronavirus main proteinase with HIV, psychotic and parasite drugs. Bioorg Med Chem 2004; 12: 2517–2521.

Zheng B, He ML, Wong KL, Lum CT, Poon LL, Peng Y, Guan Y, Lin MC, Kung HF. Potent inhibition of SARS-associated coronavirus (SCOV) infection and replication by type I interferons (IFN-alpha/beta) but not by type II interferon (IFN-gamma). J Interferon Cytokine Res 2004a; 24: 388–390.

Zheng BJ, Wong KH, Zhou J, Wong KL, Young BW, Lu LW, Lee SS. SARS-related virus predating SARS outbreak, Hong Kong. Emerg Infect Dis 2004b; 10: 176–178.

Zhi Y, Wilson JM, Shen H. SARS vaccine: progress and challenge. Cell Mol Immunol 2005; 2: 101–105.

Zhong N, Ding Y, Mao Y, Wang Q, Wang G, Wang D, Cong Y, Li Q, Liu Y, Ruan L, Chen B, Du X, Yang Y, Zhang Z, Zhang X, Lin J, Zheng J, Zhu Q, Ni D, Xi X, Zeng G, Ma D, Wang C, Wang W, Wang B, Wang J, Liu D, Li X, Liu X, Chen J, Chen R, Min F, Yang P, Zhang Y, Luo H, Lang Z, Hu Y, Ni A, Cao W, Lei J, Wang S, Wang Y, Tong X, Liu W, Zhu M, Zhang Y, Zhang Z, Zhang X, Li X, Chen W, Xhen X, Lin L, Luo Y, Zhong J, Weng W, Peng S, Pan Z, Wang Y, Wang R, Zuo J, Liu B, Zhang N, Zhang J, Zhang B, Zhang Z, Wang W, Chen L, Zhou P, Luo Y, Jiang L, Chao E, Guo L, Tan X, Pan J. Consensus for the management of severe acute respiratory syndrome. Chin Med J (Engl) 2003a; 116: 1603–1635.

Zhong NS, Zheng BJ, Li YM, Poon, Xie ZH, Chan KH, Li PH, Tan SY, Chang Q, Xie JP, Liu XQ, Xu J, Li DX, Yuen KY, Peiris, Guan Y. Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. Lancet 2003b; 362: 1353–1358.

Zhu MS, Pan Y, Chen HQ, Shen Y, Wang XC, Sun YJ, Tao KH. Induction of SARS-nucleoprotein-specific immune response by use of DNA vaccine. Immunol Lett 2004; 92: 237–243.

Ziegler T, Matikainen S, Ronkko E, Osterlund P, Sillanpaa M, Siren J, Fagerlund R, Immonen M, Melen K, Julkunen I. Severe acute respiratory syndrome coronavirus fails to activate cytokine-mediated innate immune responses in cultured human monocyte-derived dendritic cells. J Virol 2005; 79: 13800–13805.