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Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV)

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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;

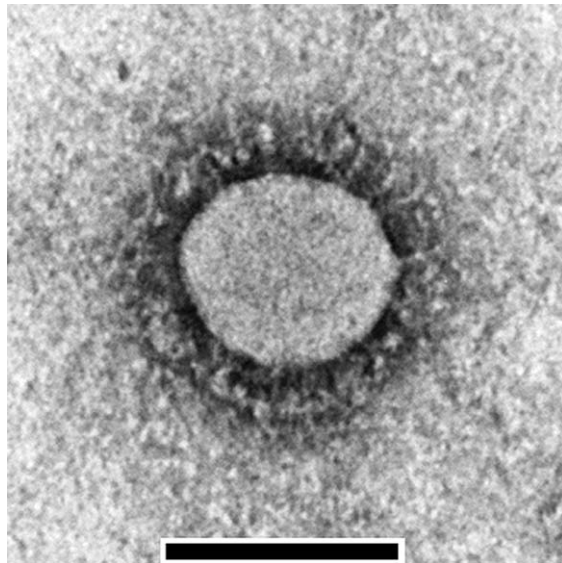


Fig. 1 Electron micrograph of SARS-CoV. The virus measures 60–120 nm in size. See text for description. Image generously provided by CDC/C.D. Humphrey and T.G. Ksiazek (US CDC Website-<http://www.cdc.gov/ncidod/sars/lab/images.htm>).

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

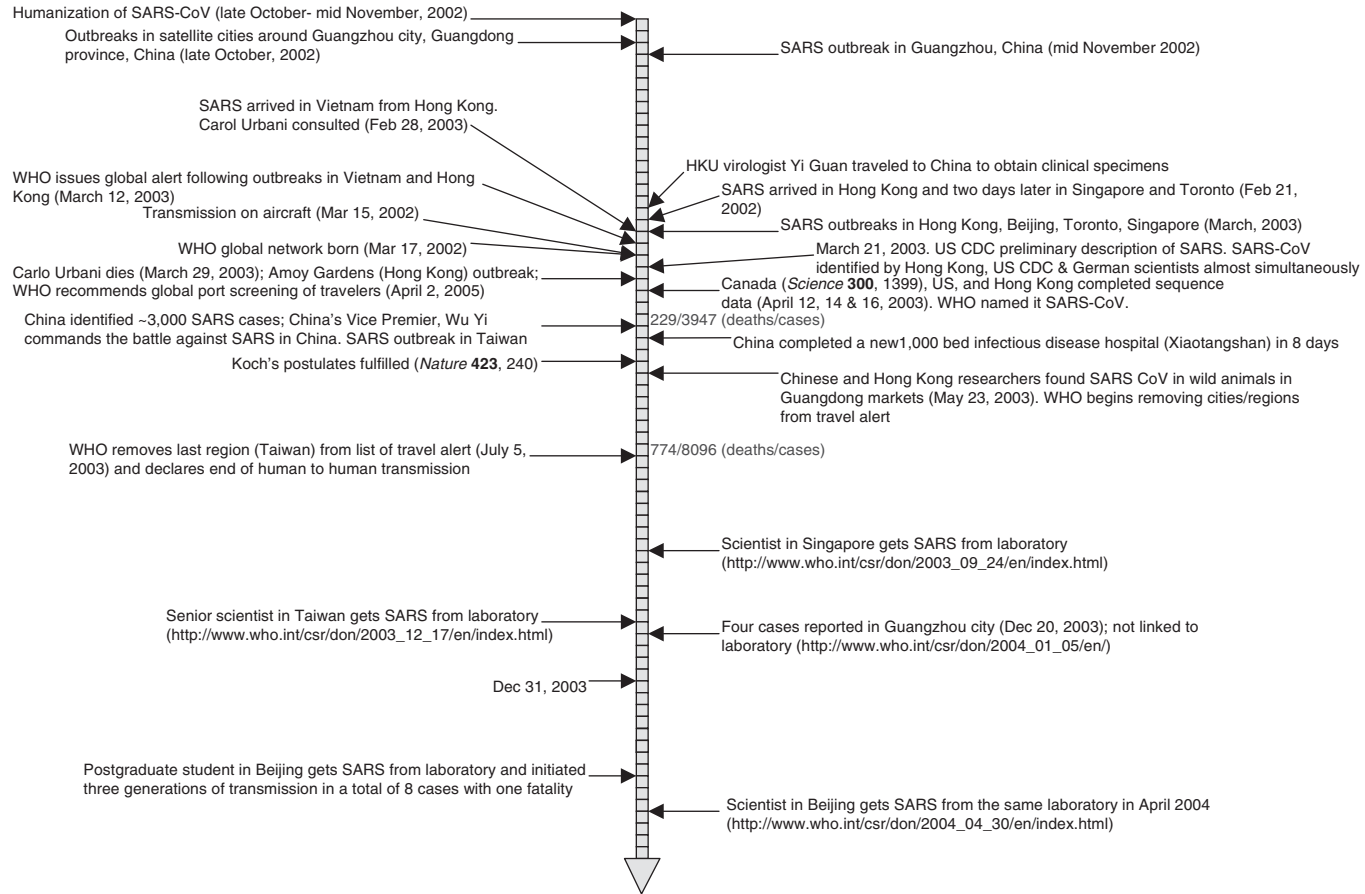


Fig. 2 Timeline of the SARS epidemic. Major events are listed from top to bottom. Each interval in the arrow represents 1 week. For information on the first weeks of the epidemic in China, consult the book by Thomas Abraham, *Twenty-First Century Plague. The Story of SARS*. The Johns Hopkins University 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).

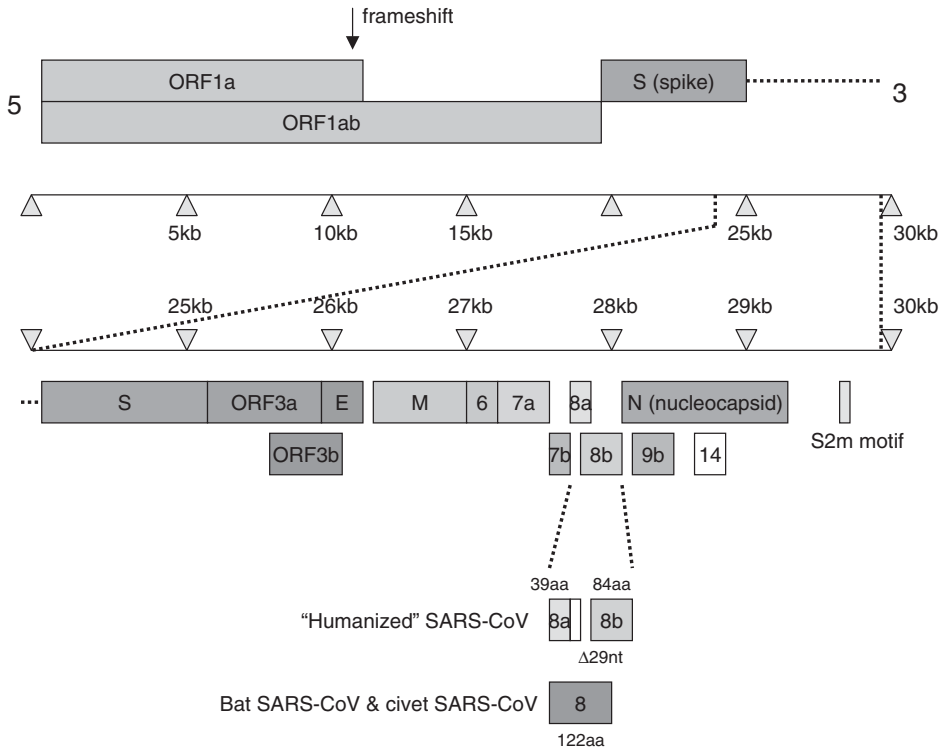


Fig. 3 SARS-CoV genome organization. The genome organization is similar to other coronaviruses with respect to overall size, the relative positions of replicase, spike, envelope, membrane and nucleocapsid genes, and certain other features (see text for details). A 29-nucleotide stretch is deleted in 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 (SE-LDI-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′ exonuclease (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 uridylylate 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 acids). 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, ORF3b, 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)- κ B 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- κ B 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- κ B 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 costimulatory 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- κ B 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 (Itayama 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 chemoattractant 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 pathogenic 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- κ B), 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 μ 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 Guangdong 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 (Reilly 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.

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