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Review

From SARS to MERS: 10 years of research on highly pathogenic human coronaviruses

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ABSTRACT

This article introduces a series of invited papers in *Antiviral Research* marking the 10th anniversary of the outbreak of severe acute respiratory syndrome (SARS), caused by a novel coronavirus that emerged in southern China in late 2002. Until that time, coronaviruses had not been recognized as agents causing severe disease in humans, hence, the emergence of the SARS-CoV came as a complete surprise. Research during the past ten years has revealed the existence of a vast pool of coronaviruses circulating among various bat species and other animals, suggesting that further introductions of highly pathogenic coronaviruses into the human population are not merely probable, but inevitable. The recent emergence of another coronavirus causing severe disease, Middle East respiratory syndrome (MERS), in humans, has made it clear that coronaviruses pose a major threat to human health, and that more research is urgently needed to elucidate their replication mechanisms, identify potential drug targets, and develop effective countermeasures. In this series, experts in many different aspects of coronavirus replication and disease will provide authoritative, up-to-date reviews of the following topics:

- clinical management and infection control of SARS;
- reservoir hosts of coronaviruses;
- receptor recognition and cross-species transmission of SARS-CoV;
- SARS-CoV evasion of innate immune responses;
- structures and functions of individual coronaviral proteins;
- anti-coronavirus drug discovery and development; and
- the public health legacy of the SARS outbreak.

Each article will be identified in the last line of its abstract as belonging to the series "From SARS to MERS: 10 years of research on highly pathogenic human coronaviruses."

Contents

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1. Introduction

"Those who cannot remember the past are condemned to repeat it" – George Santayana.

Ten years ago, a novel coronavirus causing pneumonia in humans emerged in Guangdong, China. The first known patient was a 45-year old man in the city of Foshan, who developed fever and respiratory symptoms on November 16, 2002, transmitting infection to his wife and three other family members. The second index case was a restaurant chef in Shenzhen who became ill on 10 December, returned to his home in Heyuan and transmitted infection to health care workers (HCWs) in the local hospital, including the physician who accompanied him in an ambulance to Guangzhou provincial hospital. This scenario of the emergence of clusters of cases of severe respiratory disease among family members and hospital workers, each cluster apparently going extinct after a few rounds of secondary or tertiary transmission, was played out repeatedly in subsequent weeks in a number of municipalities in Guangdong province. The index cases of many of these early case clusters were food handlers or chefs working in restaurants where a variety of exotic and game animals were slaughtered on the premises (Xu et al., 2004a,b). During subsequent weeks, the outbreak became self-sustaining, with large clusters of transmission in hospitals spilling back into the community (Table 1).

The first "super-spreading" event, which became a hallmark of the epidemiology of this disease, occurred with the hospitalization of a 44-year old man in Guangzhou on 30 January, 2003. He was to transmit infection to 19 relatives and more than 50 hospital staff. On 21 February, one of the doctors infected as part of this extended hospital outbreak traveled to Hong Kong, where he stayed one night in a hotel and was hospitalised the next day. During his stay, he transmitted infection to 16 other hotel guests and one visitor, who traveled onto their destinations, seeding outbreaks of this disease in Vietnam, Singapore, Toronto and in Hong Kong. On 12 March, following the outbreaks in mainland China, Hong Kong and Vietnam, the World Health Organization (WHO) issued a global alert about an unusual pneumonia which appeared to cause outbreaks of disease in hospitals. This led to the recognition and reporting of additional case clusters in Toronto and Singapore, prompting the WHO to issue an Emergency Travel Advisory on 15 March, providing an early case definition and naming the

Table 1
SARS and its aftermath: a chronology of events over the past ten years. Based on World Health Organization, Western Pacific Region (2006).

Date	Key events
16 November 2002	A 45-year-old man in Foshan city, Guangdong province, China develops an atypical pneumonia and infects four relatives. This is the first identified case of SARS from epidemiological investigations.
10 December 2002	A 35-year-old restaurant worker in Shenzhen develops pneumonia and 8 health care workers in contact with him become ill.
8 January 2003	A 26-year-old man working in the game animal trade in Guangxi Province (adjacent province to Guangdong) develops pneumonia and infects family members.
January 2003	Pneumonia outbreaks in Guangzhou (capital city of Guangdong Province).
23 January 2003	Guangdong Health Bureau circulates document giving case definition and control measures to health bureaus and hospitals in the province.
30 January 2003	A patient hospitalized in Guangzhou transmits infection to more than 50 hospital staff and 19 relatives, the first of many "super-spreading" events.
11 February 2003	WHO receives reports of an outbreak of respiratory disease in Guangdong, 305 cases and 5 deaths. One-third of cases are health care workers infected while caring for patients with similar illness.
21 February 2003	A doctor from Guangdong caring for patients with atypical pneumonia checks in at Hotel M in Hong Kong to attend a wedding. He had been ill since 15 February, but now deteriorates further and is hospitalized on 22 February. He infects 16 other guests and one visitor at this hotel, some of whom travel onto Vietnam, Singapore and Toronto where they initiate local clusters of transmission.
26 February 2003	A Hotel M contact is admitted to a private hospital in Hanoi and is the source of an outbreak there. Seven health care workers ill by 5 March.
4 March 2003	A Hotel M contact admitted to Prince of Wales Hospital, Hong Kong. He had been ill since 24 February, but his illness is not severe and not recognized as a possible case of the new "atypical pneumonia". By 7 March, health care workers at this hospital report a respiratory illness. Overall, he infects 50 health care workers, 17 medical students, 30 other patients and 42 visitors to the ward and 4 family members.
5 March 2003	A Hotel M contact dies in Toronto. Five family members affected.
12 March 2003	WHO issues global alert.
14 March 2003	Singapore and Toronto report clusters of atypical pneumonia. In retrospect, both groups have an epidemiological link to Hotel M. One of the doctors who had treated patients in Singapore has gone to New York and develops symptoms while traveling. He is quarantined as his flight lands in transit in Frankfurt, Germany. He has infected two family members travelling with him and one crew member.
15 March 2003	The WHO has received reports of over 150 cases of this new disease, now named Severe Acute Respiratory Syndrome (SARS). Travel advisory issued.
17 March 2003	A WHO multi-center laboratory network is established for the study of SARS causation and diagnosis.
21– 27 March 2003	A novel coronavirus is identified in patients with SARS.
14 April 2003	Mapping of the full genome of SARS-CoV is completed.
16 April 2003	WHO announces that SARS-CoV is the causative agent of SARS.
23 May 2003	A virus related to SARS-CoV is detected in animals in Guangdong.
5 July 2003	Absence of further transmission in Taiwan signals the end of the human SARS outbreak in humans.
September 2003 – February 2004	Laboratory-acquired SARS cases reported in Singapore, Taiwan and Beijing. The case in Beijing leads to limited community transmission in Beijing and Anhui.
December 2003 – January 2004	Transient re-emergence of SARS infecting humans from animal markets.
23 May 2005	The International Health Regulations are adopted by the Fifty-eighth World Health Assembly on 23 May 2005. They enter into force on 15 June 2007.
September 2012	A novel coronavirus causing respiratory disease is isolated in Saudi Arabia. Earlier cases in Jordan (April 2012) were retrospectively diagnosed. The aetiological agent is a novel human β-coronavirus, subsequently named the Middle East respiratory syndrome (MERS) coronavirus.
1 April – 23 May 2013	Outbreak of over 20 cases of MERS reported in hospitals in Al-Hasa, in eastern Saudi Arabia.
As of 1 August 2013	94 confirmed cases of MERS have been reported to WHO, leading to 46 deaths. Index cases have occurred in Jordan, Kingdom of Saudi Arabia, Qatar and the United Arab Emirates. Imported cases, sometimes with limited secondary transmission, has been reported from France, Germany, Italy, Tunesia and the United Kingdom.

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disease Severe Acute Respiratory Syndrome (WHO Western Pacific Region, 2006).

At this stage, a number of possible aetiological agents were being proposed, including *Chlamydia*, paramyxoviruses, human metapneumovirus, and coronavirus, among others. The WHO coordinated the sharing of information among laboratories, resulting in the consensus that the aetiological agent was a novel coronavirus, to be named SARS coronavirus (SARS-CoV) (Peiris et al., 2003; Ksiazek et al., 2003; Drosten et al., 2003; Kuiken et al., 2003). This WHO-mediated information sharing allowed laboratories, epidemiologists, and clinicians to achieve rapid consensus on clinical virology, patient management and virus transmission (World Health Organization Multicentre Collaborative Network for Severe Acute Respiratory Syndrome Diagnosis, 2003).

SARS spread rapidly along routes of air-travel, affecting 25 countries and territories across five continents and sickening over 8000 people, leading to the death of almost 800. Fortunately, it had an unusual feature that permitted the success of basic public health measures in controlling person-to-person transmission: the "viral load" in upper respiratory tract secretions was low in the first 5 days of illness, then increased progressively, peaking early in the second week (see Cheng et al., in this series). As a result, transmission was less common in the first days of illness, providing an opportunity for case detection and isolation to interrupt transmission. Patients were most infectious when they were hospitalised, contributing to transmission in hospitals, especially those in more developed settings where invasive and potentially aerosol-generating interventions such as bronchoscopy were more likely to be carried out.

The SARS-CoV was also unusually stable in the environment, more so than other coronaviruses or other respiratory viruses, making infection control in hospitals a challenge (see Cheng et al., in this series). It has been speculated that the enhanced stability of the SARS-CoV at lower temperatures and lower humidity, especially in air-conditioned environments, may help explain the explosive outbreaks that occurred in some regions, compared to others (Chan et al., 2011). However, as awareness grew, patients began to be identified and hospitalized earlier in the illness (Leung et al., 2004), and as effective infection control modalities were better implemented, it became possible to interrupt transmission in the community and in hospitals. Thus, on 5 July 2003, it was possible for the WHO to announce that "all known chains of human-to-human transmission of the SARS virus now appear to be broken". Such an outcome could hardly have been imagined in the dark days of March–April, when, for example, an unprecedented cluster of around 300 cases emerged over a few days in the Amoy Gardens housing estate in Hong Kong.

2. The source of the SARS-coronavirus

Once the outbreak had ended, the zoonotic source of the virus still remained to be identified. Epidemiological investigations had found that the index patients of the initial case clusters in November–December 2002 were food handlers or restaurant workers, especially those exposed to exotic wild-game animals, regarded as a winter delicacy in southern China (see Drexler et al., in this series). The increasing affluence of the past decade had led to this trade becoming highly organised and commercialised, with hundreds of diverse exotic wild-life being housed in large central markets. Investigation of these "wet markets" led to the detection of a virus closely related to SARS-CoV in a range of small mammalian species, such as Himalayan palm civets (*Paguma larvata*), raccoon dogs (*Nyctereutes procyonoides*) and others. People working in these markets had a high prevalence of antibodies to SARS CoV, even though they gave no history of having had SARS, while people working in other areas of the markets, such as vegetable stalls, or people in the community did not (Guan et al., 2003). This identified wild-game animal markets as the interface which facilitated the maintenance and amplification of SARS-CoV precursor viruses, allowing repeated exposure of the human population and leading to inter-species transmission events.

In late 2003 and early 2004, four more patients with a SARS-like illness were diagnosed. Phylogenetically, these re-emergent viruses were more closely related to viruses found at that time in game-animal markets than to those that caused the SARS epidemic the year before (Liang et al., 2004). This confirmed the contention that wet markets were the source of initial human infection and led to their closure, very likely pre-empting a re-emergence of SARS. Further work established that wild-caught palm civets showed no evidence of SARS-CoV infection, suggesting that they were intermediate, amplifying hosts, rather than the true reservoir. Novel coronaviruses, including some closely related to SARS-CoV, have been identified in bats, including insectivorous *Rhinolophid* bats (Li et al., 2005a,b; Lau et al., 2005; see Drexler et al., in this series). Subsequent work has identified the virus–receptor interactions and receptor restrictions that permit or restrict interspecies transmission events of SARS-CoV-like viruses (see the review by F. Li, in this series).

3. Antiviral therapy during the SARS outbreak

In the early phase of the epidemic, physicians had to manage severely ill patients, including some of their own colleagues, without reliable knowledge of the virus and its susceptibility to antiviral drugs. The treatment regimens that were applied will be described in detail by Cheng et al. (2013) in this series. Initially, patients were given ribavirin, a broadly active antiviral compound that is effective against some RNA viruses, such as hepatitis C virus and Lassa virus, but in retrospect showed little benefit for SARS patients. The *in vitro* activity of ribavirin on SARS-CoV replication in cell culture gave contradictory results, depending on the cell type used (Cinatl et al., 2003a; Morgenstern et al., 2005).

Many SARS patients were treated with a combination of ribavirin and corticosteroids, with mixed results (see Cheng et al., in this series). Interferon-α was administered to patients in mainland China and in Toronto, Canada. In the reports from China, a beneficial effect could not be clearly ascribed to interferon-α, as it was always used in combination with immunoglobulins or thymosin (Zhao et al., 2003). A preliminary, uncontrolled study from Toronto suggested that treatment with a combination of interferon-α and corticosteroids was superior to corticosteroids alone (Loutfy et al., 2003). In SARS-CoV-infected cell culture, interferon-α had much superior effects over IFN-α (Cinatl et al., 2003b). Polyethyleneglycol-modified interferon-α was demonstrated to protect macaques from SARS-CoV prophylactically and to reduce viral replication and tissue pathology when administered therapeutically (Haagmans et al., 2004).

Interestingly, the HIV-protease inhibitor lopinavir, often combined with ritonavir, appeared to show some benefit for SARS patients (Chu et al., 2004; see the review by Cheng et al., in this series). The antiviral effect of these compounds was also observed in cell culture. As the coronavirus genome does not code for an aspartic protease related to the HIV protease, Wu et al. (2004) tested the potency of lopinavir against the isolated SARS-CoV main protease (also called the 3C-like protease, $3CL^{pro}$), which is a cysteine protease, and found an IC_{50} of around 50 μM of around 50 cysteine protease, and found an ICavir, often combined with ritonavir, appeared to show some benefit for SARS patients (Chu et al., 2004; see the review by Cheng et al., in this series). Thot explain the observed activity in cell culture. An alternative explanation could be the anti-apoptotic activity of these HIV protease

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inhibitors (Maturrese et al., 2002). More information on the proposed binding mode of lopinavir to the SARS-CoV main protease and on attempts to improve its inhibitory potency will be presented in the review of this molecular target in this series by Zhang et al.

In mainland China, traditional Chinese medicines (TCMs) were employed in addition to one or more of the therapies described above, but it is difficult to assess their effect, as no systematic studies were carried out (see Cheng et al., in this series). The only TCM for which anti-SARS-CoV activity was demonstrated in cell culture (but at relatively high concentrations), was glycyrrhizin, a compound found in liquorice (Cinatl et al., 2003a).

4. Early attempts at rational design of anti-SARS drugs

Within three weeks of the discovery of the SARS-CoV, its complete nucleotide sequence had been determined by Marra et al. (2003) and Rota et al. (2003), making more rational approaches to antiviral drug discovery possible. For example, Luo et al. (2004) noticed some sequence similarities between the SARS-CoV nucleocapsid (N) protein and the capsid protein (CA) of HIV. The latter binds to cyclophilin A (CypA), a peptidyl prolyl *cis/trans* isomerase of the host cell, which is incorporated into the HIV particle (Gamble et al., 1996). Accordingly, Luo et al. (2004) reasoned that the N protein of SARS-CoV may also bind to CypA, and could indeed determine the K_d value to 60–160 nM. This interaction was blocked by cyclosporin A, an inhibitor of CypA, which however is used as an immunosuppressive drug. Through virtual screening techniques that employed the modelled SARS-CoV N-CypA complex as a target, Luo et al. were able to identify some other inhibitors of this interaction, but most of them showed some degree of cell toxicity. Chen et al. (2005) confirmed the interaction between CypA and the N protein and even provided evidence for an incorporation of CypA into the virion, similar to what had been shown previously for HIV-1.

However, once the SARS epidemic was over, the idea of blocking SARS-CoV replication by inhibiting CypA was not followed further, until Pfefferle et al. (2011) detected a specific interaction between CypA and the SARS-CoV non-structural protein 1 (Nsp1) (and similarly, of the HCoV NL63) by yeast-two-hybrid and other protein–protein interaction techniques (see the article by von Brunn and colleagues in this series). Consequently, they tested CsA for antiviral effects against a large range of coronaviruses and found it to be a "pan-coronavirus inhibitor" (Pfefferle et al., 2011). However, as CsA also displayed antiviral activity against the γ-coronavirus, Infectious bronchitis virus (IBV), which lacks Nsp1, it is possible that the mechanism originally proposed by Luo et al. (2004) may contribute to CsA's anticoronaviral activity (Ma-Lauer et al., 2012). Interestingly, there are anecdotal reports that cyclosporin A was occasionally used to treat SARS patients (e.g., So et al., 2003).

Efforts to design anti-SARS drugs were initiated early during the outbreak, but were hampered by a lack of structural data on molecular targets. By the end of March 2003, when the SARS-CoV was discovered as the etiological agent causing the disease, only one crystal structure of a coronavirus protein was available, that of the main protease (M^{pro} or $3CL^{pro}$) of the porcine coronavirus, transmissible gastroenteritis virus (TGEV) (Anand et al., 2002). However, in mid-May 2003, at the peak of the SARS outbreak in Beijing, the same group published the first structure of a synthetic inhibitor, a peptidyl methyl ketone, bound to the TGEV M^{pro}, as well as the structure of a second coronavirus M^{pro}, that of human coronavirus 229E, and a homology model of the SARS-CoV enzyme based on these structures (Anand et al., 2003). Furthermore, these authors suggested that AG7088 (rupintrivir), a Michael-acceptor-type inhibitor of the 3C protease of human rhinovirus, should be a good starting point for anti-SARS drug design. A little later, this compound itself was shown to have little activity against the virus (Shie et al., 2005), but derivatives of rupintrivir turned out to be quite active in virus-infected cell culture (Shie et al., 2005; Yang et al., 2005). These studies were facilitated by the determination of the crystal structure of the SARS-CoV main protease itself in June, 2003 (Yang et al., 2003). Since then, many inhibitors have been designed and synthesized that target the coronavirus M^{pro}, but few of them have undergone systematic toxicity and other preclinical studies, so that these compounds are not yet available for clinical trials in case of a recurrence of SARS (or for treatment of patients infected with the new human coronavirus, MERS-CoV, see below). The numerous studies aimed at designing inhibitors of coronavirus main proteases will be summarized by Zhang et al. in this series.

5. The SARS-CoV genome and proteome

Coronaviruses are enveloped viruses with a single-stranded RNA genome of positive polarity. This is the largest known RNA genome, with a size of 27–32 kb (27.8 kb in the case of SARS-CoV). The 14 open reading frames (ORFs) of the SARS-CoV genome code for at least 28 proteins (Fig. 1). The structural proteins are encoded in the 3′-terminal third of the genome. The spike glycoprotein (S, 77 kD) protrudes from the surface of the viral particle (hence the name "coronavirus") and is responsible for receptor binding and membrane fusion. In late 2003, angiotensin-converting enzyme 2 (ACE2) was identified as the receptor of SARS-CoV on the surface of human cells (Li et al., 2003; Wang et al., 2004). The structure of the complex between the receptor-binding domain of the SARS-CoV spike protein and ACE2 was determined by Li et al. (2005a,b), who will review this and subsequent work in this series. Proteolytic processing of the S protein, a prerequisite for membrane fusion, will be reviewed by Simmons, Pöhlmann, and colleagues. Inhibitors of the host-cell proteases involved have also been shown to prevent cell entry of SARS-CoV (see, e.g., Adedeji et al., 2013). Peptides corresponding to the heptad repeats of the trimeric S protein have been demonstrated to inhibit the fusion of the viral envelope with the host-cell membrane (e.g., Sainz et al., 2006; Liu et al., 2009).

Another most important structural protein of coronaviruses is the nucleocapsid (N) protein, which encapsulates the genomic RNA and has roles in its replication and transcription (see, e.g., Tylor et al., 2009; Grossoehme et al., 2009). Current knowledge of this protein will be reviewed by Huang and colleagues in this series. The matrix (M) protein and the envelope (E) glycoprotein complete the structural proteins, although in the case of SARS-CoV, some of the accessory proteins (see below) are also believed to be incorporated into the viral particle (see below).

ORF1 comprises about two-thirds of the SARS-CoV genome and codes for two huge polyproteins, pp1a (about 486 kD) and pp1ab (about 790 kD). Ribosome slippage at a frameshift site near the 3′-terminus of ORF1a leads to translation of the entire ORF1ab (Namy et al., 2006). The polyproteins are processed by two viral cysteine proteases, a papain-like protease (PL^{pro}, a domain in Nsp3) and the main protease (M^{pro} or $3CL^{pro}$, Nsp5) into a total of 15 or 16 non-structural proteins (Nsps). Most of them are components of the viral replication/transcription complex (RTC) but they may also adopt additional functions; for reviews, see (Sawicki et al., 2005, 2007; Pasternak et al., 2006; Masters, 2006; Perlman and Netland, 2009).

The RTC, consisting of the majority of the coronaviral Nsps and some as yet unidentified host proteins, assembles at virus-induced double-membrane vesicles (DMVs) and other unusual membrane structures, which have been derived from the ER membrane

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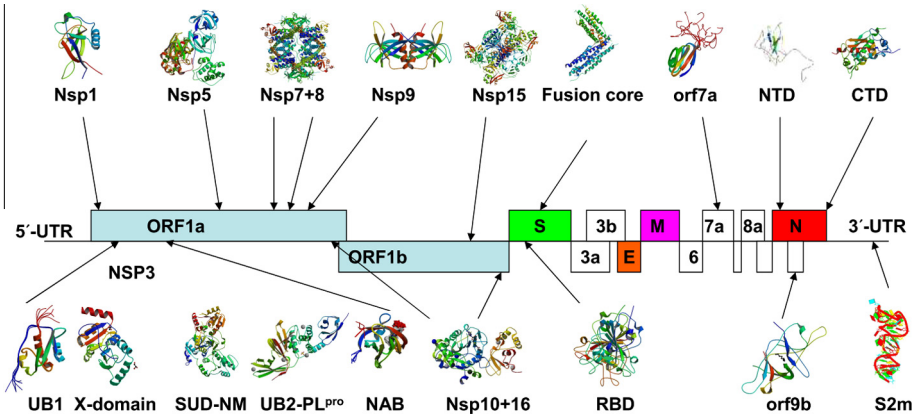

Fig. 1. Structure of the RNA genome of SARS-CoV. Three-dimensional structures are depicted for those proteins for which they are available. References to the corresponding publications and PDB codes can be found in Table 2.

Table 2
Three-dimensional protein structures for SARS-CoV and other coronaviruses.

Protein (or RNA)	Virus and PDB code	Reference
Nsp1	SARS-CoV (*2HSX; 2GDT*); TGEV (3ZBD)	Almeida et al. (2007) and Jansson (2013)
Nsp3 UB1	SARS-CoV (*2GRI*) ; MHV (*2M0I*)	Serrano et al. (2007) and Keane and Giedroc (2013)
Nsp3 PL1^{pro}	TGEV (3MP2)	Wojdyla et al. (2010)
Nsp3 X-domain	SARS-CoV (*2ACF*; 2FAV); HCoV-229E (3EWQ; 3EJG); IBV (3EWO; 3EJF; 3EKE); FCoV (3ETI; 3EW5)	Saikatendu et al. (2005), Egloff et al. (2006), Xu et al. (2009a), Piotrowski et al. (2009) and Wojdyla et al. (2009)
Nsp3 SUD	SARS-CoV (2W2G; 2WCT; *2KQV*; *2KQW*; *2JZF; 2RNK*)	Tan et al. (2009), Johnson et al. (2010a) and Chatterjee et al. (2009)
Nsp3 PL2^{pro}	SARS-CoV (2FE8)	Ratia et al. (2006)
Nsp3 NAB	SARS-CoV (*2K87*)	Serrano et al. (2009)
Nsp4-C	FCoV (3GZF) ; MHV (3VC8)	Manolaridis et al. (2009) and Xu et al. (2009b)
Nsp5	TGEV (1LVO); HCoV 229E (1P9S); SARS-CoV (1UJ1; 2BX3; 2BX4); HKU1 (3D23); IBV (2Q6D)	Anand et al. (2002, 2003), Yang et al. (2003), Tan et al. (2005), Zhao et al. (2008) and Xue et al. (2008)
Nsp7	SARS-CoV (*1YSY; 2KYS*)	Peti et al. (2005), Johnson et al. (2010b)
Nsp7 + 8 complex	SARS-CoV (2AHM); FCoV (3UB0)	Zhai et al. (2005) and Xiao et al. (2012)
Nsp9	SARS-CoV (1UW7; 1QZ8); HCoV 229E (2J97)	Sutton et al. (2004), Egloff et al. (2004) and Ponnusamy et al. (2008)
Nsp10	SARS-CoV (2FYG; 2G9T; 2GA6)	Joseph et al. (2006) and Su et al. (2006)
Nsp15	SARS-CoV (2H85); MHV (2GTH)	Ricagno et al. (2006) and Xu et al. (2006)
Nsp10 + 16 complex	SARS-CoV (2XYQ; 2XYR; 3R24)	Decroly et al. (2011) and Chen et al. (2011)
Hemagglutinin-esterase	BCoV (3CL4)	Zeng et al. (2008)
Orf7a	SARS-CoV (1XAK; 1YO4)	Nelson et al. (2005) and Hänel et al. (2006)
Orf9b	SARS-CoV (2CME)	Meier et al. (2006)
Spike RBD alone and in complex with receptor	SARS-CoV (2GHV; 2AJF); HCoV-NL63 (3KBH); PRCV (4F5C); MHV (3R4D); MERS-CoV (4L3N; 4KR0; 4KQZ; 4L72)	Hwang et al. (2006), Li et al., 2005a,b, Wu et al. (2009), Reguera et al. (2012), Peng et al. (2011), Chen et al. (2013a), Lu et al. (2013) and Wang et al. (2013)
Spike fusion core	SARS-CoV (1WYY; 2BEQ; 2BEZ; 1ZV7; 1ZVB; 1ZV8; 1ZVA; *2FXP*; 1WNC); MHV (1WDF; 1WDG); HCoV NL63 (2IEQ)	Duquerroy et al. (2005), Supekar et al. (2004), Deng et al. (2006), Hakansson-McReynolds et al. (2006), Xu et al., 2004a, b and Zheng et al. (2006)
Nucleocapsid-NTD	IBV (2C86; 2GEC; 2BXX); HCoV OC43 (4J3K); SARS-CoV (2OFZ; 2OG3; *1SSK*); MHV (3HD4)	Jayaram et al. (2006), Fan et al. (2005), Chen et al. (2013b), Saikatendu et al. (2007), Huang et al. (2004) and Grossoehme et al. (2009)
Nucleocapsid-CTD	IBV (2CA1; 2GE7; 2GE8); SARS-CoV (2CJR*; 2JW8*; 2GIB)	Jayaram et al. (2006), Chen et al. (2007), Takeda et al. (2008), Yu et al. (2006)
s2m	SARS-CoV (1XJR)	Robertson et al. (2004)

Structures elucidated by nuclear magnetic resonance (NMR) techniques are indicated by a Protein Data Bank (PDB) code in *italics*. Abbreviations: NTD, N-terminal domain; CTD, C-terminal domain; HCoV, human coronavirus; FeCoV, feline coronavirus; IBV, infectious bronchitis virus; TGEV, transmissible gastroenteritis virus. Only structures of the free proteins are listed here, inhibitor complexes are excluded. The crystal structure of the s2m element of the SARS-CoV genomic RNA is also included. Only those structures have been included for which a PDB entry is available.

(Knoops et al., 2008). In recent years, significant progress has been made in unravelling the structures of these DMVs, as will be reviewed by Snijder and colleagues in this series. After assembly of the RTC, a nested set of (sub)genomic mRNAs is synthesized and subsequently translated into the structural and accessory proteins (Sawicki et al., 2007; Pasternak et al., 2006). Finally, the structural proteins assemble into progeny virions, along with the newly synthesized genomic RNA. After budding through membranes of the intermediate ER-to-Golgi compartment (Krijnse-Locker et al., 1994), the mature virions egress from the host cell via exocytosis.

Because of their obviously essential role, the coronaviral proteases are the target of intense structural, functional, mechanistic, and inhibitor-discovery studies, and will be dealt with in separate reviews in this series by Mesecar, Baker and colleagues (on PL^{pro}) and Zhang et al. (on M^{pro}). Significant progress has also been made over the past 10 years in elucidating the structures of other Nsps, in the hope of learning something about their function. Table 2 lists the structures known to date. This effort has been made possible through several structural proteomics projects (Bartlam et al., 2005, 2007; Canard et al., 2008; Hilgenfeld et al., 2008). Overall, this approach has been quite successful, although for those proteins (mostly encoded by ORF1a) for which we know the 3D structures, the functions are still not quite clear in many cases (Nsp1, Nsp3 domains other than the PL^{pro}, Nsp9, Nsp10, also partly

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Nsp7 + 8). In contrast, for several proteins (mostly encoded by ORF1b) for which we know the functions, little structural information is available. The most painful lack of structural information concerns Nsp12, the RNA-dependent RNA-polymerase (RdRp), and Nsp13, the helicase, both of which are obvious drug targets. Progress in characterizing Nsp3 domains other than the PL^{pro} will be summarized by Lei et al., on Nsp7–Nsp10 by Xiao et al., and on Nsp12–Nsp16 by Canard and colleagues in articles in this series.

Finally, the SARS-CoV genome encodes several accessory proteins, some of which undergo rapid evolution. For example, early in the SARS outbreak ORF8 coded for one protein, but during the evolution of the virus in early 2003 it lost 29 nucleotides, and subsequently coded for two separate accessory proteins, 8a and 8b. It is thought that this event was responsible for the increased efficiency of human-to-human transmission that surfaced about the same time, triggering the epidemic (The Chinese SARS Molecular Epidemiology Consortium, 2004; Oostra et al., 2007). Structural information for SARS-CoV accessory proteins is still very scarce (see Table 2), partly due to the fact that many of these gene products are membrane proteins. In the present series of articles, the accessory proteins of SARS-CoV will be reviewed by D.X. Liu and colleagues, and evolutionary aspects of SARS-CoV will be the subject of an article by Gorbalenya and colleagues.

6. Lessons learned

The SARS epidemic vividly demonstrated that we now live in a "global village," and that an infectious disease emerging anywhere in the world has the potential to spread globally in a short period of time. One legacy of the outbreak was the formulation of the International Health Regulations (IHR) in 2005 (http://www.who.int/ihr/en/) and their acceptance by the World Health Assembly in 2007. The IHR require countries to report unusual and unexplained outbreaks of infectious disease and to develop the public health capacity to detect and respond to such diseases, when and where they occur (in this series, see the review by P. Gully). SARS and other contemporary zoonotic threats, such as H5N1 avian influenza, have highlighted the need for collaboration among those responsible for human and animal health, and the environment. This led to the formalization of the concept of "One Health", which fosters collaborative effects of multiple disciplines to attain optimal health for people, animals, and the environment. The relevant international organizations, the Food and Agriculture Organization (FAO), the World Organisation for Animal Health (OIE) and the WHO now have a formal agreement and framework within which they can coordinate activities to assess risks at the animal/human/ecosystem interface (FAO–OIE–WHO Collaboration; see http://www.who.int/influenza/resources/documents/tripartite_concept_note_hanoi_042011_en.pdf (accessed 7th August 2013)). SARS also contributed to the enhanced emphasis now being placed on better understanding of viral diversity in wildlife and the need to understand the ecological and biological bases of inter-species transmission of these pathogens.

7. Ten years after: Middle-East respiratory syndrome coronavirus (MERS-CoV)

Ten years after the SARS outbreak, it is worth recounting these events in detail and to summarise subsequent understanding of the SARS-CoV, because we continue to be confronted by novel emerging disease threats. Due to increased research efforts, two additional human coronaviruses, HCoV-NL63 and HCoV HKU1, were discovered in 2004/2005 (van der Hoek et al., 2004; Woo et al., 2005). In 2012, we saw another novel coronavirus emerge in the Middle East (Zaki et al., 2012). The novel Middle East respiratory syndrome (MERS) coronavirus is a beta-coronavirus, like the SARS-CoV but it belongs to lineage c rather than b (see Drexler et al. in this series). It causes severe pneumonia as well as renal failure, with a high fatality rate. Index cases have originated in Jordan, Qatar, Saudi Arabia, and the United Arab Emirates, while travel-associated cases have been diagnosed in France, Germany, Italy, Tunisia, and the UK. As of 1 August 2013, 94 cases have been confirmed, with 46 deaths (http://www.who.int/csr/don/2013_08_01/en/index.html).

MERS patients tend to be elderly and have other underlying illnesses. Secondary transmission has been reported in hospitals, but in one such event in France, the disease did not appear to be highly transmissible to healthy HCWs; instead, it targeted patients who were immunocompromised (Mailles et al., 2013). The largest cluster of cases was reported from a health facility in Al-Ahsa in the Eastern Province of Saudi Arabia where transmission to other patients and family members, as well as a few HCWs, has occurred (Assiri et al., 2013). In contrast to SARS, relatively few HCWs have been affected so far. Although viruses closely related to the MERS-CoV have been detected in *Pipistrellus* bats found in Europe and Africa, a more epidemiologically plausible zoonotic source and phylogenetically proximate virus remain to be identified (see Drexler et al., in this series; Annan et al., 2013). Very recently, high neutralizing-antibody titers to MERS-CoV have been detected in dromedary camels in Oman, suggesting that they may be an intermediate transmitter of the virus (Reusken et al., 2013). These events are uncannily reminiscent of the emergence of SARS in late 2002. Sero-epidemiological studies are needed to define the full extent of secondary transmission of MERS-CoV and whether the infection is more widespread in the community.

The receptor for MERS-CoV has been identified to be dipeptidyl peptidase IV (DPP4) which is expressed in the human respiratory tract and is conserved across many species, including bats (Raj et al., 2013). Biological understanding gleaned from the viral–host receptor interactions in restriction of interspecies transmission of SARS-CoV (see the review by Li, in this series) will be relevant to MERS-CoV. Two crystal structures of the complex between the receptor-binding domain (RBD) of the MERS-CoV spike protein and DPP4 have been published very recently (Lu et al., 2013; Wang et al., 2013), as has the structure of the RBD alone (Chen et al., 2013a; see the review by F. Li, in this series). A crystal structure for the MERS-CoV main protease has also been communicated (Ren et al., 2013), and an article on the macrodomains of the virus by Lei et al. is in preparation. All of these structures will be included in reviews in this series describing individual proteins of SARS-CoV and MERS-CoV.

MERS-CoV appears to replicate efficiently in human respiratory tissues (Chan et al., 2013), targeting alveolar epithelial cells and the endothelium of blood vessels in the lung, indicating a potential for disseminating beyond the respiratory tract as was seen for SARS-CoV (see Cheng et al., in this series). As with SARS-CoV, the novel coronavirus appears to avoid eliciting host interferon responses, but remain sensitive to the action of interferon (see the review by Frieman, Baric, and colleagues in this series; Chan et al., 2013; Falzarano et al., 2013; de Wilde et al., 2013). The therapeutic options tried for SARS, including interferon therapy, as summarized by Cheng et al. in this series, may be pertinent to MERS.

8. Lessons yet to be learned: the current status of antiviral therapy and vaccine development for SARS and MERS

Huge progress has been made over the past ten years in the elucidation of the functions and structures of the proteins of the SARS-CoV, and research on vaccine development has also progressed, with a number of strategies being developed and evaluated in

experimental animal models. However, it should also be noted that after 2005–2006, it became difficult to obtain funding for research on SARS-CoV in many countries, especially for efforts to discover new antiviral therapies. Similarly, there was no incentive to further develop SARS-CoV vaccines, in the absence of an overt threat to human health. Funding agencies and peer reviewers were probably short-sighted in this respect, but many virologists also failed to take seriously the threat of the re-emergence of SARS or of a SARS-like virus.

Even though many inhibitors of the SARS-CoV main protease have been designed on the basis of crystal structures (see the review by Zhang et al., in this series), few have been tested in SARS-CoV-infected cell culture, let alone in an animal model. For the other prime drug targets among the viral nonstructural proteins, the RdRp (Nsp12) and the helicase (Nsp13), the situation is even more discouraging, because it is difficult to obtain these enzymes in an active form, and numerous attempts to crystallize them have failed. Thus, the frustrating conclusion after ten years of excellent basic research on SARS-CoV is that we are still left with the therapies that showed only uncertain effects in the treatment of patients in 2003, i.e. interferon-α and lopinavir/ritonavir, which seem to be all that can be offered for the specific antiviral treatment of MERS patients. After the largely negative experiences in treating SARS patients, ribavirin and corticosteroids are no longer treatment options. There may be some hope that non-immunosuppressive derivatives of cyclosporin A, such as DEBIO-025, can be used for therapy, but such compounds remain to be tested in virus-infected cell culture. Also, a peptidic ketoamide designed on the basis of the crystal structure of the M^{pro} of the bat coronavirus HKU4, which is highly similar to the corresponding enzyme from MERS-CoV, appears to be promising, with good antiviral activity against the MERS-CoV (Lin et al., in preparation), and so do some α,β-unsaturated esters (Ma et al., in preparation). However, these compounds are only now in safety studies in animals, and it will take a long time until they can be used in the clinic. If the current MERS-CoV outbreak is over by then, there is a danger that both funding and enthusiasm for developing these and other compounds will once again wane, and we will be in the same "drug-less" situation when the next coronavirus emerges into the human population.

In conjunction with producing novel antivirals, there is a need to develop and consolidate global networks that can rapidly respond to emerging infectious disease crises such as MERS, so that novel therapeutic options may be scientifically evaluated in controlled clinical trials. Some examples of such networks are emerging, including ISARIC, the International Severe Acute Respiratory and Emerging Infection Consortium. This global initiative aims to ensure that clinical researchers have open access to the protocols and data-sharing processes needed to facilitate a rapid response to emerging diseases that may turn into epidemics or pandemics (http://isaric.tghn.org/).

As regards vaccine development, the long-lived neutralizing antibody response in those who recovered from SARS provides hope that active and passive immunization strategies are feasible, at least in principle. A number of vaccine strategies were developed and tested in laboratory animals, including recombinant vectored vaccines expressing SARS-CoV S protein, DNA vaccines, inactivated whole-virus vaccines and recombinant-protein vaccines. These studies showed that the S protein is crucial for eliciting effective protective antibody responses, and that there is a good correlation between neutralizing- antibody titers and protection from challenge in animal models. The N protein can induce antigen-specific T-cell-mediated immune responses. An inactivated whole-virus vaccine was tested in phase-1 clinical trials in China. Human monoclonal antibodies that neutralize SARS-CoV were shown to be protective for passive prophylaxis and immunotherapy in laboratory animals (Gillim-Ross and Subbarao, 2006). However, in the absence of a re-emergence of SARS, there was little incentive to pursue these initiatives, and recent years have not seen progress towards a credible SARS vaccine.

In conclusion, the SARS outbreak taught us many lessons, but that of the necessity of developing new antiviral therapies was not learned. The lack of progress we have detailed with regard to antivirals over the past 10 years is equally relevant to the development of coronavirus vaccines, for essentially the same reasons. Together with the emergence of the MERS-CoV, we hope that the articles in this series will help change the attitude of researchers and funding policy makers this time around!

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de Wilde et al. (2011) and Respiratory Syndrome Diagnosis (2003). Q5

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