Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Severe acute respiratory syndrome: identification of the etiological agent

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The severe acute respiratory syndrome (SARS) emerged in late 2002 in southern China and rapidly spread to countries around the globe. Three research groups within a World Health Organization (WHO)-coordinated network have independently and simultaneously shown that a novel coronavirus is linked to SARS. A fourth group has completed the Koch's postulates by infecting monkeys with the agent. Sequencing of the complete genome was achieved only weeks after the first isolate of the virus became available.

The severe acute respiratory syndrome (SARS) was first reported as a new disease entity to the World Health Organization (WHO) by Carlo Urbani during his work in Hanoi, Vietnam, in February 2003. He succumbed to SARS himself on 29 March 2003. Retrospectively, reports from China on cases compatible with the disease date back to November 2002. The disease involves an initial febrile phase that is followed by interstitial pneumonia, leading to respiratory distress syndrome and death in a fraction of patients. The cumulative case fatality rate at the time of writing is 8.4% [1]. On the basis of gamma-distribution models, this number has recently been corrected to values of 13.2% in patients below 60 years of age and 43.3% in those above 60 [2]. When it became clear in mid-March 2003 that hospital outbreaks with high rates of transmission had simultaneously occurred in Hanoi, Singapore, Hong Kong and Toronto (Canada), the WHO issued a global health alert and initiated studies in a network of laboratories, dedicated to clarifying the etiology of SARS [3]. Four recent articles describe how members of the network rapidly identified and confirmed a novel coronavirus as the causative agent in an independent, yet collaborative, manner [4–7].

Laboratory identification of the etiological agent

In the beginning of the investigations, known causative agents of interstitial pneumonia were sought in patients from different sites of outbreaks, and anecdotal results from some laboratories pointed to the involvement of chlamydia [5], rhinoviruses [6] and paramyxoviruses [3,8]. However, other laboratories could not confirm the presence of these pathogens in their cohorts of patients [5–7].

The initial experimental step in all three groups that later succeeded in identifying the causative pathogen was to inoculate various cell culture lines with patient specimens. Whereas cells commonly used for respiratory pathogens (e.g. LLC-Mk2, RDE, Hep-2, MRC-5, NCI-H292, HELA, MDCK, HUT-292, LLC-MK2, B95-8) yielded no indicative results, the virus could be replicated in monkey kidney cells. A group from Hong Kong was the first to observe a cytopathic effect compatible with viral growth, 2–4 days after inoculating a lung biopsy specimen and a nasopharyngeal aspirate sample on fetal rhesus kidney cells (FRhK-4) [7]. The cytopathic effect consisted of cell rounding and detachment. A specific immune response

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to infected cells was observed in patients with typical SARS but not in healthy blood donors. Similar findings were confirmed by the two other groups using African green monkey kidney (Vero) cells [5,6].

Two groups proceeded with an analysis of their cell culture supernatants by electron microscopy [6,7]. The Centers for Disease Control and Prevention (CDC) Atlanta team was the first to observe the typical electron microscopy morphology of coronaviruses, which is exemplified in Fig. 1. On the basis of conserved amino acid regions in the polymerase gene of known coronaviruses, the group designed primers directed to the polymerase gene of all coronaviruses and amplified a 405 bp fragment from the new agent. The fragment was then sequenced and compared with GenBank [6].

At the same time, groups from Europe and Hong Kong amplified the virus from their cell culture supernatants with random reverse transcriptase polymerase chain reaction (RT-PCR) techniques. One group applied differential display primers and cloned the obtained RT-PCR fragments for subsequent sequencing [7]. The other group utilized degenerated primers to produce random RT-PCR products under low-stringency conditions for direct sequencing [5]. This group was the first to report to the network a phylogenetic analysis of one of the two polymerase gene fragments they found, already suggesting that the novel agent constituted an independent genetic group within the genus *Coronavirus* [3,5]. After the genome fragments obtained in Europe and America had been exchanged through the WHO network, it was shown by amplification and sequencing of a continuous stretch of cDNA that all fragments were physically located on one viral genome [5]; the analyzed sequence bridged the communicated fragments, and the length of the product obtained (3 kb) was concordant with what would have been expected in a coronavirus genome.

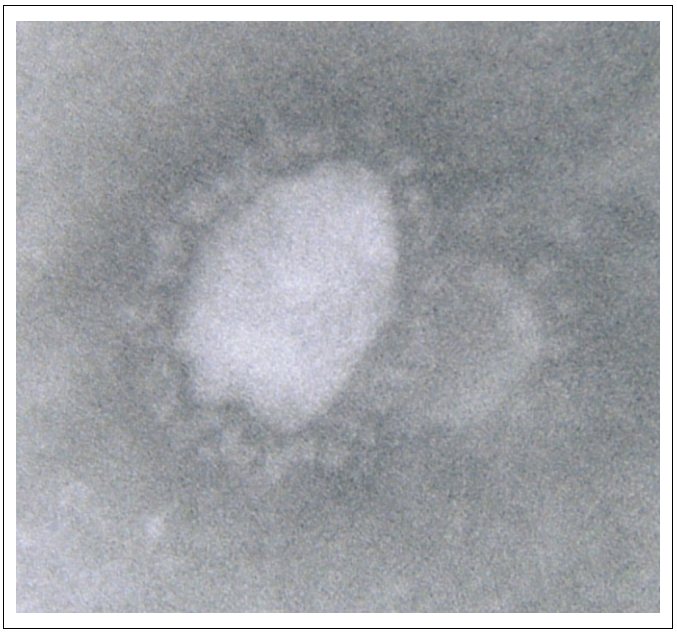

Fig. 1. Electron micrograph of severe acute respiratory syndrome (SARS)-Coronavirus, enriched by centrifugation from the supernatant of infected Vero E6 cells. The typical coronavirus morphology is represented by the large spike glycoprotein projections on the surface of the particle.

The sequence that had been obtained from the American isolate was found to be identical to the base in the European isolate. Because the European isolate was derived from Singapore and the American one from Hanoi, it was thus proven that the same virus was present in two geographically separated outbreaks of the same disease.

Linking the agent to the disease

After identification of the coronavirus in cultured specimens from single patients, all three groups devised specific RT-PCR primers based on the sequence information they had gathered. They could demonstrate the presence of the virus in large fractions of their SARS patient cohorts as well as its absence in control groups. The primer sequences were exchanged through the WHO network, and other groups confirmed the presence of the virus in their patients [8]. Immunofluorescence tests showed a specific immune response in SARS patients, but not in healthy controls (584 in total) or patients with other respiratory diseases [5–8].

The Koch's postulates were finally fulfilled by infecting cynomolgus macaques (*Macaca fascicularis*) with an isolate of the coronavirus from Hong Kong [4], to provide proof from experimental infection that the newly discovered coronavirus is the etiological agent of this disease. The typical symptoms and pathological findings observed in humans were present in the monkeys. No such symptoms were found in monkeys infected with human metapneumovirus, another prime suspect at that time, which had been additionally observed in a fraction of SARS patients [8]. Superinfection with human metapneumovirus did not aggravate the symptoms. Finally, a specific immune response to the new virus could be detected and the agent could be re-isolated from the animals.

With this wealth of data at hand only four weeks after the first cytopathic effect had been observed on a cell line, the WHO invited all laboratories participating in the SARS etiology network to Geneva on 16 April 2003. The identification of the etiological agent of SARS was formally announced and the virus was provisionally termed SARS-Coronavirus (SARS-CoV) [3].

Implications from the genome

As soon as the first isolates of SARS-CoV became available, genome sequencing was initiated in five different groups and completed almost simultaneously. Groups from Atlanta and Rotterdam communicated large fragments of preliminary sequence data during their ongoing projects, greatly accelerating the progress in other teams [9]. A group from Canada published on the internet the first complete genome of the virus. A synopsis of articles relating to SARS-CoV sequencing yields several interesting implications [9–11].

The phylogenetic analysis of all the important genes of SARS-CoV shows that it constitutes a fourth, novel, monophyletic group within the genus *Coronavirus*. There is no evidence for recombination with other coronaviruses, suggesting that the agent has evolved independently over a longer period of time. It has been reasoned that evolution has occurred in an animal

reservoir rather than in humans [11], because preliminary data suggest that the human population lacks antibodies to SARS-CoV [6,7].

The genome of SARS-CoV has no hemagglutinin esterase gene. This gene is present in most group 2 coronaviruses; a common ancestor is thought to have acquired it horizontally from influenza C virus [11,12]. Like group 1 coronaviruses, the spike (S) protein of SARS-CoV, which in many group 2 coronaviruses is cleaved into two domains (S1 and S2), lacks the typical cleavage recognition site. In common with group 3 coronaviruses, SARS-CoV has one instead of two papain-like proteases encoded by open reading frame 1a [10]. Similar to avian infectious bronchitis virus (group 3), the 3′-untranslated region of SARS-CoV contains a conserved sequence motif that is thought to have been acquired horizontally from astroviruses [11].

Two distinct genotypes of SARS-CoV have been identified by comparing complete genome sequences of isolates from different sites of the outbreak [9]. One of these genotypes is represented in all isolates that are derived from one point source of transmission, a patient who stayed at one particular hotel in Hong Kong. No difference in the clinical picture has been associated with genotypes, but a non-conservative amino acid exchange in the S1 domain of the spike protein might be a first hint for SARS-CoV responding to immunoselective pressure [9].

Conclusions

The etiology of SARS has been identified with unprecedented rapidity, facilitated by an unconventional approach of information exchange. The technical path to success was a rather conventional one. Because the agent could be cultured, a combination of classical methods (cell culture, electron microscopy, immunofluorescence) and basic molecular biology techniques (RT-PCR, random priming) has been sufficient for its identification. In other settings, where non-culturable agents were to be found, either more-sophisticated molecular techniques like subtractive amplification (representative difference analysis [13]) or more-complex combinations of classic and molecular methods have been necessary [14]. With the pathogen and its genome at hand, the necessary steps for diagnosing, treating and studying the pathogenesis of the disease can now be undertaken. The lesson learned from the identification of SARS-CoV is a general rather than a scientific one: in a situation of an impending epidemic, scientists are able to cooperate on a high level of proficiency in an efficient manner. The common trait of scientists to be competitive can be directed into a most productive direction by clever guidance, as demonstrated by the communicable disease surveillance and response team of the WHO.

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Repairing the tears: dysferlin in muscle membrane repair

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Many muscular dystrophies arise from enhanced muscle degeneration, but one muscular dystrophy subtype has now been shown to arise from defective muscle membrane repair. Mutations in the gene encoding dysferlin cause muscular dystrophy, and recent work has demonstrated a role for this protein in resealing muscle membrane tears. Thus, two broad categories of muscle membrane defects can now be defined: those with inherent instability and those with compromised repair. The latter might be relevant for muscle wasting that occurs with aging.

Muscle degeneration occurs in muscular dystrophy and also occurs with everyday use and with age. In Duchenne muscular dystrophy (DMD), mutations in the *dystrophin* gene

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