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Commentary

How the SARS vaccine effort can learn from HIV—speeding towards the future, learning from the past

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Abstract

A remarkable collaborative effort coordinated by the severe acute respiratory syndrome (SARS) team at WHO resulted in discovery of the etiologic agent of severe acute respiratory syndrome less than 2 months after the announcement of global alert. The development of a vaccine to prevent SARS should be pursued with the same urgency and cooperative spirit, as SARS is highly lethal and, if not controlled during the first few generations of transmission, is likely to become endemic in regions of the world where health-care infrastructure is underdeveloped and epidemiological control measures are weak. The scientific community already learned many important lessons from HIV vaccine development; these should be heeded. For example, consideration should be given to the development of a vaccine that will protect across regional strains of SARS, as the newly emergent coronavirus SARS-coronavirus (SARS-CoV) is proving to be variable and may be mutating in response to immune pressure. SARS-specific research reagents should also be collected and shared. These would include SARS peptides, adjuvants, DNA vaccine vectors and clinical grade viral vectors. Rapidly developing a collaborative approach to developing a SARS vaccine that will be both *effective* and *safe* is the only way to go. This article reviews parallels between HIV and SARS and proposes an approach that would accelerate the development of a SARS vaccine.
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Keywords: SARS; Coronavirus; HIV; Vaccine

1. Introduction

Due to a remarkable effort coordinated by the severe acute respiratory syndrome (SARS) team at WHO, an understanding of the clinical and epidemiological characteristics and etiology of severe acute respiratory syndrome was achieved less than 2 months from the announcement of the outbreak in China. As a result of this groundbreaking international collaboration we now know that the SARS epidemic is due to a human coronavirus (HCoV) infection, now named SARS-coronavirus (SARS-CoV). Several SARS-coronavirus genomes have been mapped and the functions of at least some of the proteins have been determined [1,2]. These genomes are now available on GenBank, making it possible for laboratories around the world to begin the process of building a safe and effective SARS vaccine.

Although there is much to learn about SARS many lessons can already be drawn from our experience with HIV. The first lesson to keep in mind concerns strain variability. SARS, like HIV, is a RNA virus whose replication is error-prone. Studies of 14 separate SARS strains that emerged from a single point source suggest a pattern of evolution in response to immune pressure that is much like the evolution of HIV. Some forms of coronavirus—murine hepatitis virus in particular—are known to mutate to escape from host immune response. Will clades and strains of SARS emerge as they have for HIV? If so, will SARS vaccine researchers be able to develop a vaccine that adequately addresses this problem? HIV vaccine developers were slow to address HIV variability; scientists involved in the SARS vaccine effort must address this issue more promptly.

More important, the HIV vaccine development effort initially focused on replicating the approach that had been used to develop Hepatitis B vaccine development, which was to clone and express the surface protein of the virus. This simplistic approach to a complex virus was recently proven to be a failure [3]. Will scientists once again pursue “quick and easy solutions” in the hopes of stimulating a protective antibody response despite existing evidence that coronavirus vaccines (for animals) based on the S or spike surface protein have largely been ineffective?

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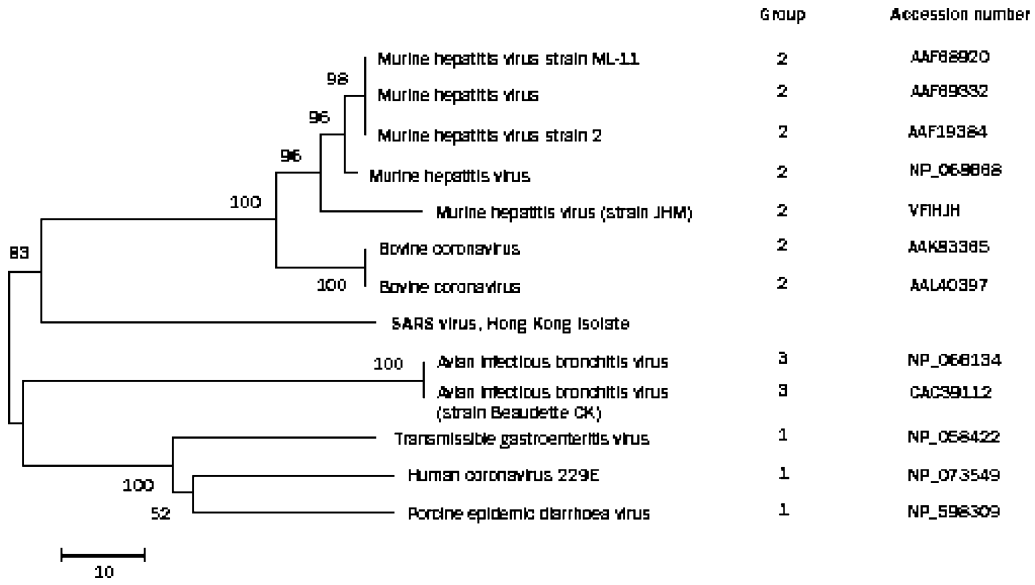


Fig. 1. Coronavirus classification (reprinted with permission from The Lancet 2003, 361 (9371), 1779–1785 [7]). The Coronaviridae family contains the genuses coronavirus and torovirus. The genus coronavirus is broken down into three groups. SARS-CoV has been assigned to a fourth group. Group 1 includes canine coronavirus (CCV), feline coronavirus (FIPV), human coronavirus 229E (HCoV-229E), porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus (TEGV). Group 2 species are bovine coronavirus (BCoV), human coronavirus OC43 (HCoV-OC43), murine hepatitis virus (MHV), porcine hemagglutinating encephalomyelitis virus (HEV) and rat coronavirus (RCV). Group 3 species are avian infectious bronchitis virus (IBV) and Turkey corona-virus (TCoV).

In order to best define an effective SARS vaccine approach, it is important to consider the correlates of protection from coronaviruses in animal models and any evidence for immunity that may be emerging from clinical experience. Little is known, as yet, about the human immune response to SARS-CoV but much can be learned from examining the immunology of other coronaviruses and the history of coronavirus vaccines. We must also examine the host–pathogen interaction and ask whether specific antigens (proteins derived from the pathogen) or host responses, such as antibodies and T cell response, provide any insight into the type of vaccine that should be developed.

SARS, like HIV, has the potential to set off a global pandemic. In this article, we argue that there is much to be learned from the past—both in terms of building a cooperative approach in order to expedite the development of an effective vaccine and in terms of the scientific knowledge that has emerged as a result of this collaborative process [4]. There is no better time to begin working on a new SARS vaccine than now. It is imperative to capture this moment, when the virus is relatively new, before clades and strains of this highly virulent airborne virus emerge.

2. Overview

2.1. Virology

Coronavirus is a common and worldwide pathogen that infects a variety of mammals and birds. This virus has been classified into three groups; SARS-CoV has now been assigned to a fourth group because it is unlike other existing coronaviruses [5]. Coronavirus experts participating in a meeting on SARS recently held at the NIH[1] reached the following consensus: that the SARS-CoV is ‘most like’ Group 2, which includes bovine and murine coronaviruses [6].

Coronaviruses are positive-strand, single-strand RNA viruses that belong to the order Nidovirales, which also includes the families Arteriviridae, Coronaviridae and Roniviridae. The Coronaviridae family contains the genuses coronavirus and torovirus. The genus Coronavirus is broken down into three groups. SARS-CoV has been assigned to a fourth group (see Fig. 1 [7]).

Human coronavirus infections (HCoV) are seasonal. They generally manifest as wintertime respiratory infections and enteric infections (mostly in infants <12 months). Adult infections are less common than infections among children. HCoV can also rarely cause neurological syndromes. Reinfection appears to be common, even though antigenic variation is limited (in the S or spike protein). Given that the full extent of HCoV variation has yet to be determined, it is possible that variations in other (non-structural) proteins may allow escape from immunity, a phenomenon that has been observed with other coronaviruses such as murine hepatitis virus [8,9].

In contrast with HCoV, re-infection with SARS-CoV appears to be relatively rare, and in at least one case, according to investigators reporting from Hong Kong at the recent NIH

[1] The National Institute Of Allergy and Infectious Diseases Symposium entitled “SARS Developing a Research Response” was held 30 May 2003 at the Natcher Conference Center National Institutes of Health, Bethesda, Maryland.

conference on SARS, the reoccurrence was associated with a milder form of the illness. While reinfection in the case of common-cold coronavirus HCoV-229E implies either that immunity is incomplete or that antigenic variation impedes the development of immunity, the low rate of reinfection (based on available data) that has been observed with SARS and the high rate of recovery from acute illness in the absence of effective medical therapy (approximately 80% of young adults do recover) suggest that *protection from disease is achievable*. This raises hope for the development of a vaccine.

However, escape mutations in response to immune pressure have been observed, as with HIV [8,9] and selected point mutations are known to causes major shifts in the pathogenicity of coronaviruses, as well as in tissue specificity [10]. The fact that the virus is famously difficult to replicate without error and that it is able to tolerate large reductions as well as point mutations in its genome suggest that variation in the sequence of SARS can be expected as the epidemic continues to move through human populations (much as has been observed with HIV). Accordingly, the evolution of strain variability and the identification of conserved or cross-strain epitopes will need to be monitored during the development of a SARS-CoV vaccine, just as the emergence of new strains is monitored for influenza.

2.2. Genome

The genome for the coronavirus believed to be responsible for the global outbreak of SARS has been sequenced [11]. Amplification of short regions of the polymerase gene (the most strongly conserved part of the coronavirus genome) by RT-PCR and nucleotide sequencing has revealed that the SARS virus is a novel coronavirus that has not previously been present in human populations. This conclusion is confirmed by serological investigations [12]. SARS-coronavirus appears to be a novel coronavirus that falls midway between the cat and human species, although many Coronavirus experts believe that it is closer to Group 2 coronavirus (Bovine CoV and MHV).

The SARS-CoV genome is 29,727 nucleotides in length and the genome organization is similar to that of other coronaviruses. Eleven open reading frames corresponding to known coronavirus proteins include the polymerase protein (polymerase 1a, 1b), spike protein (S), small membrane protein (E), membrane protein (M) and nucleocapsid protein (N) have been identified (Fig. 2 [7]). These 11 open reading frames are believed to encode as many as 23 separate proteins with both known and unknown functions. Most of the non-structural proteins seem to be encoded in the first half of the genome, whereas most of the structural proteins such as spike, membrane, envelope and nucleocapsid are located in the second half of the genome.

Mutations in the genome have already been described [7–13]. According to a recent report comparing 14 SARS genomes linked by a common point source, despite the limited amount of time that elapsed between primary transmission at Hotel M and the development of secondary cases in Singapore (less than 2 months), a total of 94 amino acid sequence mutations in the RNA polymerase, the spike glycoprotein, the membrane nucleocapsid, and several uncharacterized putative proteins were identified [5] (Fig. 2).

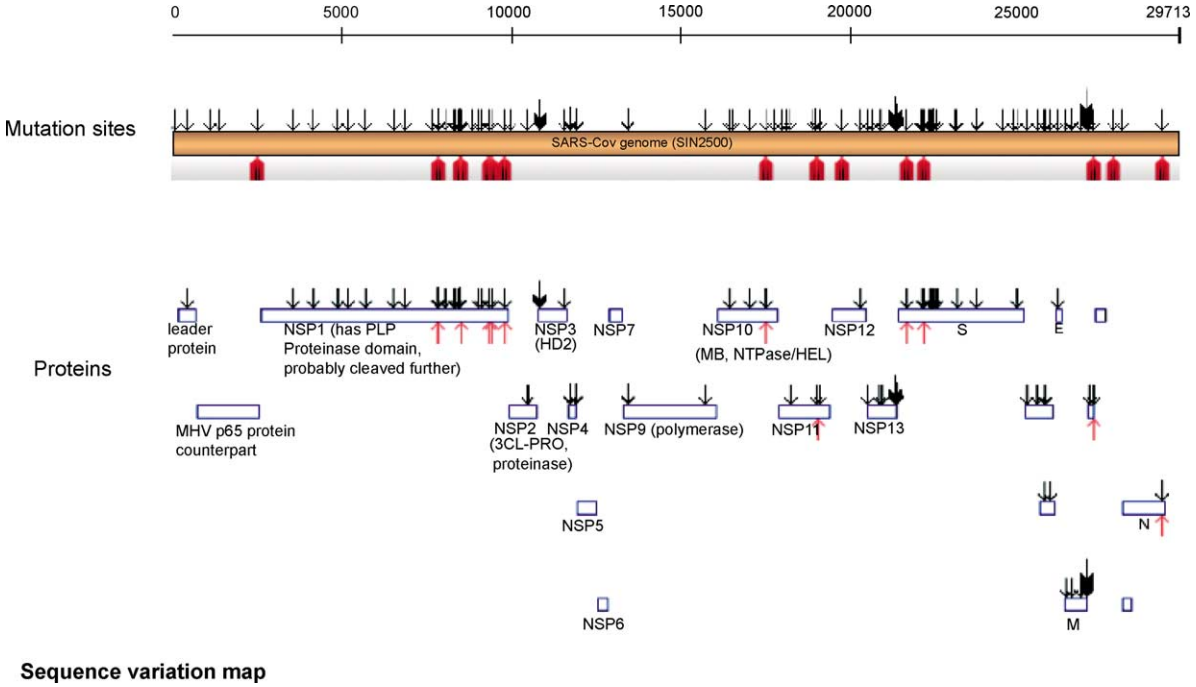


Fig. 2. SARS evolution (reprinted with permission from The Lancet 2003, 361, 1779–1785). Mutations mapped in 14 SARS isolates associated with a single point source (Hotel M). Upward arrows indicate recurrent variations. Black arrows indicate variations in a single isolate.

One particular amino acid change in position 22,222 of the genome (an isoleucine residue changed to a threonine, a non-conservative amino acid change in the S1 region of the spike protein) has been observed. This modification, which occurred during the carriage of SARS-CoV from Hong Kong (Hotel M) to Singapore by one patient may have allowed the virus to escape immune response. Mutations have previously been observed in the MHV spike protein, in response to pressure from cell-mediated immune response [13].

Mutation of a large genome (30,000 bases) in response to immune pressure is an ominous sign for the future of vaccine development against SARS-CoV and indicates that we may see a degree of variation in the SARS genome that matches, or exceeds, HIV.

2.3. Clinical presentation

The most common reported clinical presentation of SARS, based on hospitalized patients, is fever (94%). More than half of persons affected (51–72%) report general influenza-like symptoms, chills, malaise, gastrointestinal symptoms, loss of appetite, and myalgia [14]. The mean incubation period of SARS is estimated to be between 4 and 6 days. The case fatality rate has been as high as 15% for patients younger than 60 years and can be higher than 50% for patients aged 60 years or older. Nearly 40% of patients developed respiratory failure that requires assisted ventilation [15]. Most cases occur within 7 days of infection (in Guandong, 70 cases occurred within 7 days after a single point-exposure) [16].

Unfortunately, given that the clinical manifestations of SARS do not allow ready distinction from other common respiratory viral infections, the diagnosis of SARS may become more difficult as epidemiological links with travel to selected areas diminish over time. Rapid and accurate diagnostic tools will be critical in the management of this epidemic. Once these tools are available, we may discover, as we did with HIV, that there are indeed many more persons who are infected yet do not manifest the illness—in other words, hospitalized patients may represent just the ‘tip of the iceberg’.

Diagnostic tests for SARS are currently limited to antibody assays (dependent on the development of Ab thus detection of infection is only possible 10–12 days following infection) and PCR assays (sensitive but technically difficult in some settings, and likely to be false-negative in the early days of the infection).

Transmission occurs by droplet transmission and may also occur by the fecal oral route, although food is not, as yet, a suspected means of transmission in the current outbreak. SARS-CoV can be detected at the time of clinical presentation, persisting throughout acute illness and decreasing during convalescence. According to reports from investigators speaking at the NIH conference on SARS, respiratory secretions are still PCR positive (but no virus has been cultured) more than 40 days after presentation, as are stool samples.

In animal coronavirus infection, the S protein (spike), a prominent transmembrane protein (two domains, S1 and S2) determines the species and tissue specificity of each coronavirus [17]. Modifications of S are associated with changes in the type of cell targeted by the virus. Epithelial cells (such as those that line the respiratory tract) appear to represent the main target for SARS, although hepatic, renal, cardiac and ophthalmic tissues may be infected by other coronaviruses. Coronaviruses have also been reported to infect macrophages. In the course of HCoV respiratory infections, growth of the virus in infected cells appears to be localized to the epithelium of the upper respiratory tract. SARS-CoV, in contrast, appears to affect cells lining the lower respiratory tract.

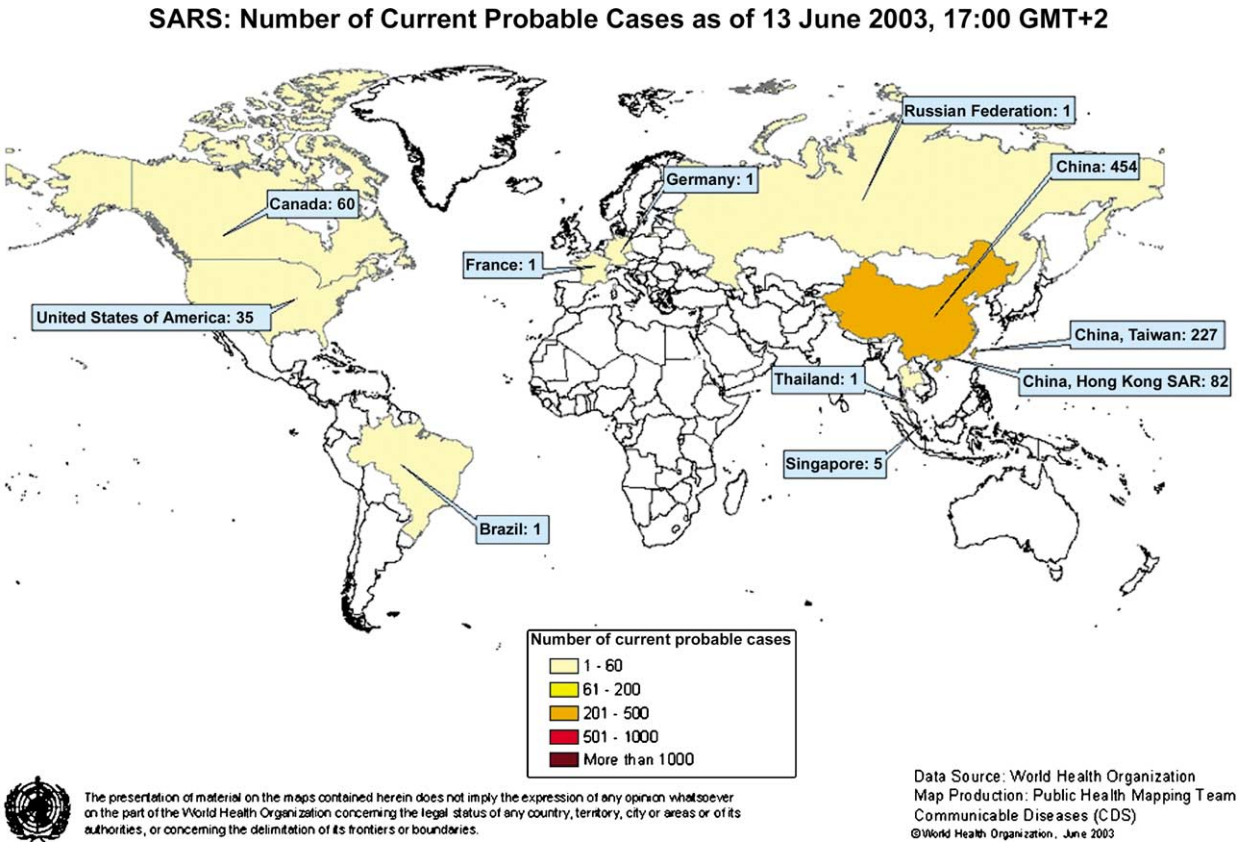
Even though growth in human cell lines is not a common feature of coronaviruses, SARS virus can be grown in Vero cells (a fibroblast cell line); SARS-CoV infection results in a cytopathic effect and budding of coronavirus-like particles from the endoplasmic reticulum within infected cells [12]. SARS-CoV does not infect mice, nor, according to CDC investigators speaking at the NIH conference, does it infect SCID. The host target cell range and target cell receptor for SARS-CoV remain to be discovered at this time. Finally, due to the limited host cell range of the virus, no adequate animal model for SARS-CoV has been found.

3. Host–pathogen relationship

3.1. Transmission

The current SARS outbreak is believed to have originated in China in late 2002. WHO issued a global alert about the emergence of severe acute respiratory syndrome on 12 March 2003. While only five deaths were reported at that time, there was immediate and grave concern about the rapid spread of the disease. Within 2 weeks, 11 countries had also reported cases for a total of 569 cases (or 264 not including Chinese cases). In response to these developments, CDC issued interim guidance concerning infection control precautions in the health-care and community setting and raised concerns about international travel. Despite these precautions, the numbers of persons and countries affected continued to climb. As of 26 May 2003, the WHO reported a cumulative total of 8202 probable cases with 725 deaths from 28 countries (http://www.who.int/csr/en/) (Fig. 3). Health-care workers are increasingly affected by SARS; the most recent re-emergence of SARS in Canada is clearly linked to nosocomial transmission [18].

While local epidemics can be contained with sound barrier precautions, there is good reason to believe that SARS will become endemic and that outbreaks will recur during the Fall and Winter transmission seasons. One reason to believe that SARS will become endemic is that transmission of the virus continues unabated in regions outside of larger cities in China. As of 30 May 2003, nine regions in China



qualified as having category B (second generation) or C (epidemiologically unlinked) transmission.[2] Pattern C transmission is mainly occurring in regions outside larger cities such as Inner Mongolia. As of this writing, no cases of SARS had been described in Africa, India or South America, but the impact of the arrival of SARS (which spreads by both airborne and fecal-oral routes) in regions of the world where crowded cities, poor sanitation and fragile health-care infrastructures are a fact of life, is likely to be revealed over the next few months. As with HIV, uncontrolled transmission can be expected to occur in regions of the world where access to health-care is poor and understanding of the basic precepts of infectious disease transmissibility is extremely limited.

Transmission in pattern C cases may be due to asymptomatic shedders of the SARS virus. Canadian and Chinese physicians reported to assembled scientists at the NIH conference on SARS that a least 14% of contacts in Canada and a number of cases in Hong Kong showed evidence of infection (seroconversion) but did not report symptoms. Thus, the number of total infections, if asymptomatic shedders are included, may be much higher than previously reported. Furthermore, serologic evidence of infection has been observed in a range of small animals, suggesting that animal reservoirs may exist, which would mean that complete eradication of the virus by quarantine may be difficult to achieve.

3.2. Immunity to SARS

No information is available on the immune correlates of protection to SARS. We are thus forced to extrapolate from available clinical information and from information gleaned from the study of coronavirus infections in animals.

Several aspects of the clinical presentation of SARS deserve mention. First of all, 70–80% of individuals recover from infection, and there appears to be a relationship between recovery and decline in viral load [7]. This suggestion that immunity can be acquired offers great promises for the development of an effective vaccine. Second, anecdotal reports of individuals who have seroconverted but never reported an illness indicate that immunity to infection can occur rapidly after exposure in some individuals and, moreover, that illness may be preventable. Third, elderly individuals have been observed to have the highest mortality rate (greater than 50%). Higher mortality rates in older individuals may be due to their limited ability to generate new B and T cell responses and contain new infections. In summary, immunity to SARS is achievable and illness may be prevented.

Lastly, the clinical presentation of SARS is subacute, unlike most infectious diseases, except for chronic viral

[2] Pattern B: More than one generation of local probable SARS cases, but only among persons that have been previously identified and followed-up as known contacts of probable SARS cases. Pattern C: Local probable cases occurring among persons who have not been previously identified as known contacts of probable SARS cases.

Date (2003)	Cases	Deaths
March 17th	167	4
March 26th	1323	49
April 26th	4836	293
May 26th	8202	725

Source: Communicable Disease Surveillance & Response (CSR)
http://www.who.int/csr/alertresponse/en/

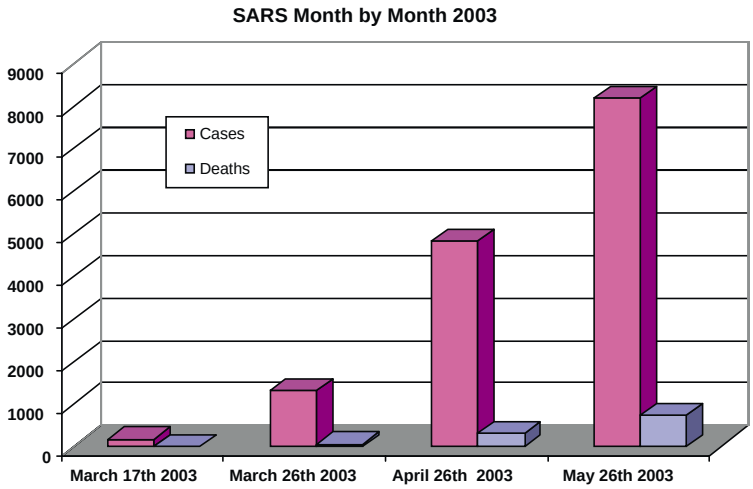


Fig. 3. SARS Epidemiology. Source: Communicable Disease Surveillance & Response (CSR), http://www.who.int/csr/alertresponse/en/.

diseases like HIV and hepatitis C. SARS is characterized by a long (5–10 day) prodromal phase, a gradual climb in the viral load, peaking around day 10, and a decline in viral load by day 15 as symptoms improve—for those individuals who recover from the illness. Studies contrasting patients who do recover with those who do not have not yet been performed.

3.3. Immunopathogenesis

Immune responses may contain and possibly also exacerbate SARS. While T cell responses would be expected to be present as soon as day 2–4 of infection, antibody seroconversion has been shown to occur at around day 10, when symptoms can exacerbate. Antibody-mediated exacerbation has been observed in two separate coronaviral diseases in animals (Feline Infectious Peritonitis and Bovine coronavirus-associated Shipping Fever, see below). Currently, the only accepted clinical intervention in SARS has been aggressive suppression of local immune responses using high dose and inhaled steroids. Ribivarin, which has also been used, is known to modulate immune responses and may have no direct effect on the SARS virus itself.

Both cell-mediated and humoral immune responses have been associated with exacerbations of disease in some coronavirus infections—these adverse effects must be carefully considered when designing a vaccine. For example, in the case of murine hepatitis virus (MHV) (a Group 2 coronavirus) disease, T cell response is protective, but T cells of both types (CD4 and CD8) have been implicated in the demyelination of the brain and spinal cord following infection with neurotropic MHV [19,20]. Antibody response may also be detrimental in the setting of infectious bronchitis virus (IBV) in chickens—although incomplete, there is some evidence that birds that have low level humoral immunity (as measured by antibody titers in tears) do worse than those with higher levels of humoral immune response [21]. There is also a link between humoral responses to bovine viral diarrhea virus and the development of “Shipping Fever” (attributed to a bovine respiratory coronavirus) in cattle feedlots [22].

3.4. Correlates of protection

Correlates of protection from coronavirus disease have been studied in animal models. Coronavirus experts have emphasized that there is a wide range of coronavirus diseases, and that the clinical manifestations of the disease and the correlates of protection can vary widely between pathogens and between animal models.

Both humoral and cellular immune responses contribute to protection against coronavirus disease in animal models. In some settings, antibodies and T cells contribute to exacerbation of the pathology although the mechanism by which this occurs is not well understood, and the role of CD8 versus humoral responses is hotly debated. In coronavirus infections such as MHV and BoCV (Bovine Coronavirus, also Group 2), T cells are critical to protection against illness. Both CD4 and CD8 T cells (T helper and cytotoxic T cells or CTL, respectively) are involved [23,24]. T cell immunity is also required for protection against porcine endemic diarrhea virus (PEDV) [25], and both humoral and cell-mediated immunity are involved in the immune response to turkey coronavirus infection (TCoV) [26]. Infectious bronchitis virus is a devastating disease for chicken producers and has been the focus of many vaccine studies. Both CD8 and CD4 T cells appear to be involved in the protective immune response to IBV [24–27].

It is also important to note that cross-virus T cell immunity does exist (as has been described between Japanese encephalitis virus (JEV) and West Nile virus (WNV) in animal models [28]). For example, pigs who have had been exposed to porcine respiratory coronavirus (PRCV) are protected against virulent transmissible gastroenteritis virus (VTE), in a protective immune response that is attributed to T cells (protection correlates with T cell proliferation) [29].

There is evidence in MHV that T cells are required to *eradicate* infection while antibody is involved in reducing viral load during acute infection [30]. These differences may be related to the ability of some coronaviruses to form syncitia (note that syncitia-like giant cells were observed in lung tissue specimens derived from SARS patients [31]). If cell-to-cell transmission via syncitia formation also occurs in SARS, eradication of the SARS-CoV may also not be achieved by humoral response alone, therefore T cell immunity may be required to clear infection [23].

3.5. Immune escape

A number of laboratories have mapped T cell epitopes in coronavirus infections. For example, CD4 T cell epitopes have been identified in the M and nucleocapsid proteins of MHV [32,33], in the nucleocapsid of an avian coronavirus [34] and in TGEV of swine [35]. Persistent infection in the case of MHV seems to be due to CTL escape (mutations in the viral genomes that abrogate the ability of the cellular immune system to stimulate CTL response). Mutation occurs, even in the MHV spike protein, in response pressure from cell-mediated immune response [13]. Escape from CTL response may allow MHV to persist in the CNS [8,9,36]. The MHV-specific CTL response is polyclonal, but CTL escape occurs nonetheless [37,38].

This type of mutation in response to immune pressure that has been observed for some coronaviruses is highly reminiscent of CTL escape that has been observed in the course of HIV infection. Since SARS-CoV, like HIV, is an RNA virus that has an error-prone replication mechanism, there is reason to be concerned that variants of SARS-CoV that escape cellular immune response may also evolve. Mutations in the SARS-CoV genome in the S protein, which may have occurred in response to immune pressure, have been described [7].

4. Vaccines against coronaviruses

The likelihood of developing a safe and effective vaccine against SARS-CoV is high. Most people do become immune and survive the disease. Once we define the correlates of immunity from survivors, we should be able to make a vaccine. One approach to the development of a safe and effective vaccine would be to map T cell epitopes that are recognized by SARS survivors and conserved across strains of SARS-CoV to clone these epitopes into a vaccine vector and to demonstrate proof of immunogenicity in human immune system (HLA) transgenic mice prior to testing the vaccine in SARS patients and normal volunteers.

4.1. Live attenuated vaccines

Live attenuated coronavirus vaccines can be made by deletion in “group specific genes” which are specific for each of the groups—deleting these genes does not alter replication but does attenuate the virus [39]. Live attenuated IBV vaccine has been combined with inactivated IBV with good success in broiler chickens [29]. Live attenuated vaccines have also been made using a more traditional method, which consists in passaging the virus obtained from one species of animal (pigs) in cells derived from another species (cattle) [40].

In general, the fact that live attenuated vaccines are significantly more effective than whole killed vaccines in animal disease suggests that the development of cell-mediated immunity is critical to protection against coronaviruses. However, there is great concern that the vaccine strain could recombine with wild type circulating strains [41] and it is unlikely that the FDA will approve the use of live attenuated SARS-CoV vaccines in humans without extensive evidence that recombination and reversion to virulence do not occur.

4.2. Whole killed and subunit vaccines

Whole killed vaccines are very common in the animal food industry, as they are generally safe and cheap to produce. “Autologous” vaccines, which are vaccines that are developed for a virus circulating in a specific herd of cattle or group of chickens, are often used, and limited licenses are issued to allow the use of these vaccines in a specific geographic region. This approach has been used with coronaviruses as both BoCV and IBV are significant pathogens for cattle and chickens. A whole killed bovine coronavirus vaccine has been developed for cattle that appeared to be safe and effective [42]. An inactivated canine coronavirus vaccine is available to protect against canine coronavirus in young dogs; however, as it is not clear that this killed vaccine can protect against different strains of canine coronavirus, the vaccine is underutilized in the veterinary industry [43]. In a comparison of vaccines to prevent IBV, live attenuated vaccine appeared to be much more effective than whole killed vaccine [44].

4.3. Recombinant subunit vaccines

Recombinant subunit vaccines (use of molecular biology techniques to produce large quantities of recombinant viral proteins) are likewise expected to be limited in their ability to protect against viruses that have evolved in the human population under immune pressure, causing significant variation in the viral genome. While recombinant technology will certainly permit the rapid development of a SARS spike protein-based vaccine—efforts are already underway to produce such proteins safely—the need for T cell immunity (as has been shown with MHV and BoCV, two related viruses) and the description of S1 region variability so early in the epidemic point to the fact that this approach will need to be supplemented by a cell-mediated immunity-directed vaccine, which is relevant to the whole of the SARS genome.

4.4. Recombinant vectored vaccines

A recombinant fowlpox containing the S1 gene of IBV has been produced and was shown to be relatively protective against IBV [45]. A fowlpox virus expressing C terminal nucleocapsid protein of IBV has also been developed. This construct protects against challenge by homologous strain and some cross-strain protection has also been observed. This cross-strain protection may have been due to effective presentation of conserved CTL epitopes by the viral vector [46]. A DNA vaccine containing the nucleoprotein gene of porcine transmissible gastroenteritis virus (PTGV) has been used to vaccinate against gastroenteritis—both humoral and cell-mediated immune responses are induced [47].

Note that WHO officials have raised concern that coronavirus variation will make it difficult to develop a single vaccine based on the spike protein. As with flu, it may become necessary to update the vaccine, which would require similar global surveillance.

The "vectored" approach (DNA or viral vector) is currently highly favored by coronavirus experts. The DNA prime and adenovirus or MVA boost approach that is currently being explored for HIV vaccine development might be an avenue worth exploring for the SARS pathogen. A multi-valent (multi gene), mixed humoral and cell-mediated approach is strongly supported by coronavirus experts since all of the animal models point to the involvement of more than one arm of the immune system.

4.5. Epitope-based vaccines

Epitopes are easily delivered in the context of DNA or viral vectors. An epitope-driven approach to coronavirus vaccine development has already been attempted with some success [48]. One advantage of the epitope based approach is that any region of the SARS-CoV genome that may be similar to self and therefore associated with a potential for autoimmune effects—can be eliminated. The epitope-based approach would avoid any possibility of reversion to virulence and may be better able to avoid the type of vaccine-induced enhancement of disease that appears to be associated with some vaccines against BoCV, FIPV and MHV. A cell-mediated immunity-directed vaccine that is highly unlikely to recombine (this statement is most true with epitope-based vaccines and least true with live attenuated vaccines) could also be useful for the treatment of SARS. This method has been used for some HIV vaccines and could provide an ethically appropriate avenue for the testing of a safe, epitope-based SARS vaccine in humans.

Variation leads to escape. As subunit or whole killed vaccines can only prime against one strain of virus, their efficacy against variant viruses may be lower. If SARS evolves as HIV has, the development of viral variability may preclude the use of a single strain of SARS-CoV in a vaccine. One means of solving the problem of variability is to search for conserved epitopes that are conserved across strains of SARS-CoV. Epitopes that are highly mutable will not protect against SARS-CoV as the virus evolves, which may make the selection of epitopes that are highly conserved, as with the some HIV vaccines under development [4] very relevant here.

5. Conclusion

There is much to be learned from the HIV vaccine effort and from previous studies of coronavirus vaccines in animals that is applicable to SARS. There are worrisome similarities between SARS-CoV and HIV; both are RNA viruses and able to mutate under selection pressure in the host; and coronaviruses are especially prone to mutation and recombination. SARS-CoV sequencing has already uncovered some evidence of mutation in response to immune pressure. Selecting multiple highly conserved "Achilles' heel" epitopes from the SARS immunome, as has been done for at least one HIV vaccine prototype [4], will permit the development of a vaccine that will remain relevant as SARS-CoV evolves.

Both humoral and cell-mediated immune responses may be required to protect against SARS-CoV. There is, however, some evidence from animal studies that humoral response to some coronaviruses can contribute to host pathology, and the role of antibody in the exacerbation of disease in SARS has yet to be defined. Until the role of antibody is clarified, vaccines directed at eliciting humoral immunity need to be evaluated for their potential to exacerbate disease. Cross-reactive T cell responses have also been implicated in host pathology (in the MHV model). Therefore, developing a T cell-directed vaccine that is composed of T cell epitopes that are in no way cross-reactive with the host may be the safest approach. Consideration might also be given to combining a T cell-directed vaccine with a whole recombinant vectored spike protein vaccine, should both vaccines prove safe and efficacious.

The immune responses of recovering and convalescent SARS patients provide the most important measure of immunity to SARS vaccine design. The immune responses of these patients should be used to direct the development of the vaccine.

In conclusion, the development of a SARS vaccine should be pursued with the utmost urgency, as SARS is highly lethal and is likely to become endemic in underdeveloped regions of the world. A pathway to vaccine development that will take advantage of the collective expertise of the vaccine development community, making available vaccine components such as vectors and delivery vehicles that have been previously evaluated in human clinical trials, will expedite the development of a vaccine. Research reagents should also be collected and shared. These would include SARS peptides, adjuvants, DNA vaccine vectors and clinical grade viral vectors. We have much to learn from the HIV vaccine effort, which has been marred by grandstanding and poor cooperation. Rapidly developing a collaborative approach to developing a SARS vaccine that will be both *effective* and

safe is the best way to address this newly emergent infectious disease.

6. Resources

The National Institute of Allergy and Infectious Diseases (NIAID), NIH is providing at access to several hundred SARS microarrays at no charge to the scientific community.

Distribution of the GeneChip® brand SARS Array, made by Affymetrix Inc., will be coordinated by the NIAID's Pathogen Functional Genomics Resource Center (PFGRC).

SARS arrays can be requested via a Web-based application process found at http://www.niaid.nih.gov/dmid/genomes/pfgrc/default.htm.

Videos documenting the morning and afternoon plenary sessions of the National Institutes of Health's research colloquium on Severe Acute Respiratory Syndrome on 30 May 2003 are available at http://www.videocast.nih.gov/ram/sars053003.ram (Real Player is required).

All of the Powerpoint presentations from the morning and afternoon sessions are also posted on NIAID's web site. They can accessed at http://www.niaid.nih.gov/sars_meeting.htm.

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