Severe Acute Respiratory Syndrome (SARS)

Development of Diagnostics and Antivirals

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ABSTRACT: The previously unknown coronavirus that caused severe acute respiratory syndrome (SARS-CoV) affected more than 8,000 persons worldwide and was responsible for more than 700 deaths during the first outbreak in 2002–2003. For reasons unknown, the SARS virus is less severe and the clinical progression a great deal milder in children younger than 12 years of age. In contrast, the mortality rate can exceed 50% for persons at or above the age of 60. As part of the Sino-European Project on SARS Diagnostics and Antivirals (SEPSDA), an immune phage-display library is being created from convalescent patients in a phagemid system for the selection of single-chain fragment variables (scFv) antibodies recognizing the SARS-CoV.

KEYWORDS: SARS; phage display; antibodies; antiviral agents

INTRODUCTION

In February 2003, the new and previously unknown deadly coronavirus causing severe acute respiratory syndrome (SARS-CoV) was brought to the attention of the World Health Organization (WHO) by Dr. Carlo Urbani and his colleagues.1

The SARS virus originated in the province of Guangdong in southern China in November 2002 where it initially was thought to cause atypical pneumonia.2 However, within a short time the virus spread to Hong Kong, Singapore, Vietnam, Canada, the United States, Taiwan, and several European countries.

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Concerted efforts of the scientific community led to a very rapid identification of a novel coronavirus as the etiological agent of SARS and the full genome sequencing of the virus.\textsuperscript{3–9} The SARS-CoV genome is $\sim$30 kb in length and contains 14 potential open reading frames (ORFs).\textsuperscript{7,10}

According to the WHO, the SARS-CoV affected more than 8,000 individuals worldwide and was responsible for over 700 deaths during the first outbreak in 2002–2003. For reasons unknown the SARS virus is less severe and the clinical progression a great deal milder in children younger than 12 years of age.\textsuperscript{11} In contrast, the mortality rate was highest among patients $>$65 years\textsuperscript{12} and can exceed 50\% for persons at or above the age of 60 years.\textsuperscript{13} In Hong Kong, where 298 people died from SARS, the mortality rate for children (age 0–14 years) was 0\%. On the other hand, 63.9\% of the cases were in persons older than 65 years, most of whom showed a history of chronic disease (http://www.hku.hk/ctc/sars_hk_23). At present no experimental evidence can explain the observed age distribution. However, it should be noted that a recently discovered coronavirus strain, NL63, exhibits a markedly different age distribution with regard to clinical symptoms.

The coronaviruses are a group of viruses that have a crown-like (coronal) appearance. The SARS-CoV are positive-strand RNA viruses and the virion consists of a nucleocapsid core encapsulated by the three envelope glycoproteins: spike (S), membrane (M), and envelope (E) proteins that are common to all members of the genus. The RNA is packaged by the nucleocapsid (N) protein into a helical nucleocapsid.\textsuperscript{14} The SARS virus N protein has only a 32\% identity with the other known coronaviruses and has been suggested to be a major immunogen.\textsuperscript{15} The S protein is known to be a major target of the cellular immune response and plays an important role in the initial roles of infection.

**SARS DIAGNOSIS AND THERAPY**

Continuous work is being performed to find an early diagnosis and therapy of SARS. Several different approaches have been taken. Diagnosis has been performed by serologic testing using indirect fluorescent antibody or enzyme-linked immunosorbent assays (ELISA) specific for SARS-CoV antibody.\textsuperscript{16,17} Detection of the SARS-CoV itself has been done using clinical specimens of serum, nasal secretions, and stool. This was done through viral isolation and electron microscopy, viral culture, or reverse transcription polymerase chain reaction (RT-PCR) to test for viral RNA.\textsuperscript{3,8}

Neutralizing antibodies (NAbs) have been found against the SARS virus. One of the targets of these NAbs is the S glycoprotein, especially toward the metallopeptidase angiotensin-converting enzyme 2 (ACE2) binding domain [S(318-510)].\textsuperscript{18} NAbs have been selected against the ACE2 domain.\textsuperscript{19,20}
Within the SEPSDA consortium significant progress has been made, with regard to both a structural understanding of the SARS-CoV\textsuperscript{21–23} and the development of putative therapies.\textsuperscript{24}

To further increase the understanding of viral biology and to help devise novel therapies, an effort to harvest the protective immunity generated by convalescent patients has been initiated. Antibodies against the SARS proteins can be obtained in different ways. The most commonly used are hybridomas to make monoclonal antibodies\textsuperscript{25} and immunization followed by collection of antisera.\textsuperscript{26} Selections using phage display have been performed, selecting scFv antibodies from semi-synthetic (nonimmune) libraries\textsuperscript{27–29} and with immune library.\textsuperscript{30}

The first time a fusion protein was displayed on the surface of filamentous bacteriophages was in 1985 by G.P. Smith,\textsuperscript{31} who showed that foreign DNA fragments could be inserted in the middle of gene III to create a fusion protein. The phage particle displays a protein or peptide on the surface and carries the gene for the displayed protein or peptide inside the particle, giving a linkage between phenotype and genotype.\textsuperscript{31} This allows for the selection of phage displaying a protein or peptide with affinity for a given target, and at the same time the gene encoding the protein or peptide is co-selected. In this way, it is possible to screen millions of different displayed proteins or peptides.

In 1990 a single-chain antibody fragment (scFv) was displayed on the surface of filamentous bacteriophage for the first time by McCafferty \textit{et al.}\textsuperscript{32} In 1991 came the first publications displaying libraries of fragment antigen-binding (Fab) fragments on gIIIp\textsuperscript{33,34} and on gVIIIp.\textsuperscript{35} Subsequently, better and larger libraries have been constructed and used for selection of antibodies against numerous different antigens.

The creation of large phage-display libraries gives the potential of isolating human antibodies against most antigens,\textsuperscript{36} making it possible to bypass both hybridoma technology and immunization.\textsuperscript{37} In addition, because no immune system is involved in the selection of antibodies by phage display, it is possible to select antibodies against toxic compounds, lethal pathogens, and highly conserved antigens.\textsuperscript{38}

In general two kinds of antibody libraries can be created from donors: immune and naïve (nonimmune) libraries. The immune libraries are made from immunoglobulin (Ig) variable region (V) genes derived from immunized donors.\textsuperscript{37} An immune library is biased toward a specific antigen, which leads to the selection of higher-affinity antibodies (compared to naïve libraries of the same size). The naïve libraries are made from Ig V genes derived from non-immunized donors or from synthetic V-genes.\textsuperscript{36} The naïve library is unbiased and can therefore select antibodies against virtually any antigen.
Creation of immune phage-display libraries for immunized donors has shown a particular efficiency in selecting neutralizing antibodies (NABs) against different viruses, for example, rabies, varicella-zoster, hepatitis A and E, measles, and respiratory syncytial virus.

The use of phage display seems ideal for the selection of antibodies against the SARS-CoV. Creating an immune library, based on peripheral blood lymphocytes from convalescent patients in a phagemid system, would provide the possibility of developing an early diagnosis and therapy of SARS. NAb has been selected, but an early and fast diagnosis is still important, should another outbreak occur.

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