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.[3–9] The SARS-CoV genome is ~30 kb in length and contains 14 potential open reading frames (ORFs).[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.[11] In contrast, the mortality rate was highest among patients >65 years[12] and can exceed 50% for persons at or above the age of 60 years.[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.[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.[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.[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.[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)].[18] NAbs have been selected against the ACE2 domain.[19,20]

SINO-EUROPEAN PROJECT ON SARS DIAGNOSTICS AND ANTIVIRALS (SEPSDA)

Within the SEPSDA consortium significant progress has been made, with regard to both a structural understanding of the SARS-CoV[21–23] and the development of putative therapies.[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[25] and immunization followed by collection of antiserum.[26] Selections using phage display have been performed, selecting scFv antibodies from semi-synthetic (nonimmune) libraries[27–29] and with immune library.[30]

The first time a fusion protein was displayed on the surface of filamentous bacteriophages was in 1985 by G.P. Smith,[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.[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 *et al*.[32] In 1991 came the first publications displaying libraries of fragment antigen-binding (Fab) fragments on gIIIp[33,34] and on gVIIIp.[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,[36] making it possible to bypass both hybridoma technology and immunization.[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.[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.[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.[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,[39] varicella-zoster,[40] hepatitis A[41] and E,[42] measles,[43] and respiratory syncytial virus.[44]

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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REFERENCES

1. World Health Organization. 2003. Severe acute respiratory syndrome (SARS): multi-country outbreak. http://www.who.int/csr/don/2003_03-16/en/.
2. Rosling, L. & M. Rosling. 2003. Pneumonia causes panic in Guangdong province. Br. Med. J. **326:** 416.
3. Drosten, C. *et al*. 2003. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N. Engl. J. Med. **348:** 1967–1976.
4. Ruan, Y.J. *et al*. 2003. Comparative full-length genome sequence analysis of 14 SARS coronavirus isolates and common mutations associated with putative origins of infection. Lancet **361:** 1779–1785.
5. Peiris, J.S. *et al*. 2003. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet **361:** 1319–1325.
6. Rota, P.A. *et al*. 2003. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science **300:** 1394–1399.
7. Marra, M.A. *et al*. 2003. The genome sequence of the SARS-associated coronavirus. Science **300:** 1399–1404.
8. Ksiazek, T.G. *et al*. 2003. A novel coronavirus associated with severe acute respiratory syndrome. N. Engl. J. Med. **348:** 1953–1966.
9. Fouchier, R.A. *et al*. 2003. Aetiology: Koch's postulates fulfilled for SARS virus. Nature **423:** 240.
10. Thiel, V. *et al*. 2003. Mechanisms and enzymes involved in SARS coronavirus genome expression. J. Gen. Virol. **84:** 2305–2315.
11. Leung, C.W. *et al*. 2004. Severe acute respiratory syndrome among children. Pediatrics **113:** e535–e543.
12. Liang, W. *et al*. 2004. Severe acute respiratory syndrome, Beijing, 2003. Emerg. Infect. Dis. **10:** 25–31.
13. Donnelly, C.A. *et al*. 2003. Epidemiological determinants of spread of causal agent of severe acute respiratory syndrome in Hong Kong. Lancet **361:** 1761–1766.

14. TAN, Y.J., S.G. LIM & W. HONG. 2005. Characterization of viral proteins encoded by the SARS-coronavirus genome. Antiviral Res. **65:** 69–78.
15. KROKHIN, O. *et al*. 2003. Mass spectrometric characterization of proteins from the SARS virus: a preliminary report. Mol. Cell Proteomics **2:** 346–356.
16. LI, G., X. CHEN & A. XU. 2003. Profile of specific antibodies to the SARS-associated coronavirus. N. Engl. J. Med. **349:** 508–509.
17. CHEN, W. *et al*. 2004. Antibody response and viraemia during the course of severe acute respiratory syndrome (SARS)-associated coronavirus infection. J. Med. Microbiol. **53:** 435–438.
18. WONG, S.K. *et al*. 2004. A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. J. Biol. Chem. **279:** 3197–3201.
19. SUI, J. *et al*. 2004. Potent neutralization of severe acute respiratory syndrome (SARS) coronavirus by a human mAb to S1 protein that blocks receptor association. Proc. Natl. Acad. Sci. USA **101:** 2536–2541.
20. YI, C.E. *et al*. 2005. Single amino acid substitutions in the severe acute respiratory syndrome coronavirus spike glycoprotein determine viral entry and immunogenicity of a major neutralizing domain. J. Virol. **79:** 11638–11646.
21. ANAND, K. *et al*. 2003. Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs. Science **300:** 1763–1767.
22. ZHAI, Y. *et al*. 2005. Insights into SARS-CoV transcription and replication from the structure of the nsp7-nsp8 hexadecamer. Nat. Struct. Mol. Biol. **12:** 980–986.
23. YANG, H. *et al*. 2003. The crystal structures of severe acute respiratory syndrome virus main protease and its complex with an inhibitor. Proc. Natl. Acad. Sci. USA **100:** 13190–13195.
24. CHEN, L. *et al*. 2005. Cinanserin is an inhibitor of the 3C-like proteinase of severe acute respiratory syndrome coronavirus and strongly reduces virus replication in vitro. J. Virol. **79:** 7095–7103.
25. WEN, K. *et al*. 2004. Preparation and characterization of monoclonal antibodies against S1 domain at N-terminal residues 249 to 667 of SARS-associated coronavirus S1 protein. Di Yi Jun Yi Da Xue Xue Bao. **24:** 1–6.
26. LIN, Y. *et al*. 2003. Identification of an epitope of SARS-coronavirus nucleocapsid protein. Cell Res. **13:** 141–145.
27. LIU, H. *et al*. 2004. Recombinant scFv antibodies against E protein and N protein of severe acute respiratory syndrome virus. Acta. Biochim. Biophys. Sin. (Shanghai). **36:** 541–547.
28. ZHONG, X. *et al*. 2005. B-cell responses in patients who have recovered from severe acute respiratory syndrome target a dominant site in the S2 domain of the surface spike glycoprotein. J. Virol. **79:** 3401–3408.
29. VAN DEN BRINK, E.N. *et al*. 2005. Molecular and biological characterization of human monoclonal antibodies binding to the spike and nucleocapsid proteins of severe acute respiratory syndrome coronavirus. J. Virol. **79:** 1635–1644.
30. DUAN, J. *et al*. 2005. A human SARS-CoV neutralizing antibody against epitope on S2 protein. Biochem. Biophys. Res. Commun. **333:** 186–193.
31. SMITH, G.P. 1985. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science **228:** 1315–1317.
32. MCCAFFERTY, J. *et al*. 1990. Phage antibodies: filamentous phage displaying antibody variable domains. Nature **348:** 552–554.
33. BARBAS, C.F. III *et al*. 1991. Assembly of combinatorial antibody libraries on phage surfaces: the gene III site. Proc. Natl. Acad. Sci. USA **88:** 7978–7982.

34. HOOGENBOOM, H.R. *et al*. 1991. Multi-subunit proteins on the surface of filamentous phage: methodologies for displaying antibody (Fab) heavy and light chains. Nucleic Acids Res. **19:** 4133–4137.
35. KANG, A.S. *et al*. 1991. Linkage of recognition and replication functions by assembling combinatorial antibody Fab libraries along phage surfaces. Proc. Natl. Acad. Sci. USA **88:** 4363–4366.
36. MARKS, J.D. *et al*. 1991. By-passing immunization: human antibodies from V-gene libraries displayed on phage. J. Mol. Biol. **222:** 581–597.
37. CLACKSON, T. *et al*. 1991. Making antibody fragments using phage display libraries. Nature **352:** 624–628.
38. HUST, M. & S. DUBEL. 2004. Mating antibody phage display with proteomics. Trends Biotechnol. **22:** 8–14.
39. KRAMER, R.A. *et al*. 2005. The human antibody repertoire specific for rabies virus glycoprotein as selected from immune libraries. Eur. J. Immunol. **35:** 2131–2145.
40. KAUSMALLY, L. *et al*. 2004. Neutralizing human antibodies to varicella-zoster virus (VZV) derived from a VZV patient recombinant antibody library. J. Gen. Virol. **85:** 3493–3500.
41. KIM, S.J. *et al*. 2004. Neutralizing human monoclonal antibodies to hepatitis A virus recovered by phage display. Virology **318:** 598–607.
42. SCHOFIELD, D.J. *et al*. 2003. Monoclonal antibodies that neutralize HEV recognize an antigenic site at the carboxyterminus of an ORF2 protein vaccine. Vaccine **22:** 257–267.
43. DE CARVALHO NICACIO, C. *et al*. 2002. Neutralizing human Fab fragments against measles virus recovered by phage display. J. Virol. **76:** 251–258.
44. NGUYEN, H. *et al*. 2000. Efficient generation of respiratory syncytial virus (RSV)-neutralizing human MoAbs via human peripheral blood lymphocyte (hu-PBL)-SCID mice and scFv phage display libraries. Clin. Exp. Immunol. **122:** 85–93.