題名:A dominant antigenic epitope on SARS-CoV spike protein identified by an avian single-chain variable fragment (scFv)-expressing phage

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摘要:Abstract
Severe acute respiratory syndrome (SARS) is a newly emergent human disease, which requires rapid diagnosis and effective therapy. Among antibody sources, immunoglobulin Y (IgY) is the major antibody found in chicken eggs and can be used as an alternative to mammalian antibodies normally used in research and immunotherapy. In this study, phage-expressing chicken monoclonal scFv antibody was chosen and characterized with phage display antibody technology. Truncated fragments of SARSCoV spike protein were cloned in pET-21 vector and expressed in BL-21 Escherichia coli (E. coli) cells. After purification, the purity of these recombinant spike proteins was examined on SDS-PAGE and their identity verified withWestern blot analysis using anti-his antibodies and sera from convalescent stage SARS-CoV-infected patients. Using these bacteria-derived proteins to immunize chickens, it was found that polyclonal IgYantibodies in the egg yolk and sera were highly reactive to the immunogens, as shown by Western blot and immunocytochemical staining analysis. A phage displaying scFv library was also

established from
spleen B cells of immunized chicken with 5 107 clones. After four panning cycles, the eluted phage titer showed a 10-fold
increase. In sequence analysis with chicken germline gene, five phage clones reacted, with large dissimilarities of between 31 and
62%, in the complementarity-determining regions, one dominant phage 4S1 had strong binding to fragment Se-e, located between
amino acid residues 456–650 of the spike protein and this particular phage had significantly strong binding to SARS-CoV-infected
Vero E6 cells. Based on the results, we conclude that generating specific scFv-expressing phage binders with the phage display
system can be successfully achieved and that this knowledge can be applied in clinical or academic research.