利用噬菌體重組基因體庫製造 **SARS-CoV** 核殼蛋白抗原之雞抗體片段

Chicken single chain variable fragment recognizing SARS-CoV Nucleocapsid protein antigen using phage display antibody technology

中文摘要

嚴重急性呼吸道症候群(severe acute respiratory syndrome, SARS)在2003年在世界各地造成重大傷亡，目前已知爲新型冠狀病毒 SARS-CoV 所引起，而研究顯示病毒顆粒中的核殼蛋白(nucleocapsid; N protein)可以幫助病毒入侵宿主細胞，造成感染。所以快速檢驗出以及有效的治療方法是必須的。本篇研究目標是利用重組表現五個不同長度片斷的核殼蛋白來免疫雞隻，並藉由噬菌體展現技術(phage display technique) 來建立重組抗體基因庫 (antibody library) 篩選對於核殼蛋白具有特異性結合力的單株抗體片段 (scFv, single-chain variable fragment)。此實驗使用 pGEX 質體，分別在大腸桿菌 BL-21 細胞中表現五個病毒核殼蛋白(nucleocapsid protein) 的 DNA 片段。利用 GST SepharoseTM 4B 做純化後，可以利用 coomassie blue 染色的聚丙醯氨膠體電泳分析以及西方墨點法(western blot) 來辨認蛋白。而之後將進一步將純化後的 GST- nucleocapsid 片段蛋白與佐劑混合均勻，以皮下注射方式注入實驗雞隻大腿部位，每週一次、連續四週。純化雞蛋內的多株抗體 (poly-IgY)，並利用酵素連結免疫吸附分析法（enzyme-linked immunosorbent assay, ELISA）以及西方墨點法來確認免疫過後雞隻所誘發出的抗體可辨認 GST-nucleocaspsid 片段蛋白。並以嗜菌體展現（phage display）技術，找尋出對於核殼蛋白可辨認具有特異性結合力的單株抗體片段(scFv, single-chain variable fragment) 。未來希望能進一步運用這些 scFv 抗體片段應用到臨床及實驗研究上，並成爲專一性的診斷試劑。

英文摘要

Severe acute respiratory syndrome (SARS) has led to serious casualties worldwide in year 2003, which can be attributed to a SARS-associated coronavirus or SARS-CoV. Research revealed that nucleocapsid protein (N protein) in the virus particles allows it to invade the host cells and cause infection. An effective and rapid diagnosis or treatment of SARS should be developed. In this study, 5 truncated SARS-CoV nucleocapsid DNA fragment were cloned into pGEX vector and expressed as GST-nucleocapsid in the E. coli BL-21 cells. We used GST Sepharose 4B to purify GST-nucleocapsid protein and then use coomassie blue stain and western blot for the detection of purified GST-nucleocapsid fusion protein. The purified

GST-nucleocapsid fusion protein was then mixed with adjuvant and injected intramuscularly into Leghorn chicken. Polyclonal IgY antibodies were purified from the immunized chicken and examined by enzyme-linked immunosorbent assay (ELISA) and western blot analysis. Our study aims to use a recombinant 5 nucleocapsid protein of different length fragment to immunize chicken and constructed an antibody library by phage display technology. By screening of a scFv (single-chain variable fragment) phage library, the nucleocapsid protein specific scFv antibodies was isolated. This will generate specific binding scFv that can identify N protein by phage display. These scFv antibody molecules can be further applied clinically and experimentally for future studies.