以 LAE 技術製備抗 CXCR3 和 CXCL10 具單株性質的多株抗體以用於白斑症基礎研究和臨床治療

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摘要:

白斑病是一種皮膚疾病，全球約有七千萬人染病，不僅影響病患的外觀，也會影響病患與人互動，進而造成病患心理問題。白斑病因素主要可能是自體免疫、環境因素、情緒壓力、飲食習慣和曝曬所造成。因為病因仍不甚清楚，所以目前沒有有效根治策略和藥物或處理方式。從自體免疫相關研究上顯示此病與一些免疫相關蛋白有關，例如 CXCL10 趨化因子(chemokine)和 CXCR3 受體(receptor)。動物相關研究中顯示白斑症小鼠的皮膚和血液中的 CXCL10 含量較高。若以 CXCL10 的中和性抗體處理白斑症小鼠，可以讓小鼠重新覆色(repigmentation)，並減輕白斑症症狀。此外，$CXCR3^{-/-}$缺失的小鼠經抗體處理也可減緩其症狀。這些數據都建議 CXCL10/CXCR3 的抗體有其臨床上應用的價值。

本研究的目的，是使用 LAE (Linear Array Epitope)技術，產生含 CXCL10 和 CXCR3 某一特殊胺基酸序列之重複性抗原相對的 DNA 片段。將此片段接上蛋白質表現質體，並送入細菌表達需要的蛋白(命名為 LAE 抗原)。大量表達和純化此抗原後，經免疫紐西蘭白兔收取血清以生產類單株的多株抗體。所得之抗體進行會先進行基礎的分生與生化測試後，不僅可供相關學術研究使用，也會評估臨床治療之可行性。

目前實驗進度已成功透過 LAE 技術，獲得 CXCL10 和 CXCR3 重複性目標 DNA 片段，並且接上蛋白質表現質體送入細菌得到 LAE 抗原的 DNA 序列。目前正將 LAE 抗原送去定序確認。接著，會於細菌中表達進行並純化此抗原，並免疫紐西蘭白兔收取血清，並將評估抗體的專一性和靈敏度等生化性質，並進行純化以供後續相關研究。

Production of monoclone-like polyclonal antibodies against CXCR3 and CXCL10 for academic study and clinical treating of vitiligo by LAE technology

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Abstract：

Vitiligo, a skin disease, is estimated to affect 70 million people worldwide. This disease not only affects the patient's appearance, but also affects the patient's interaction with other people, in turn leading to psychological problems. Factors of vitiligo include autoimmunity, environmental factors, emotional stress, dietary habits and exposure to sun. Because the cause of vitiligo is still unclear, there is no effective cure strategy, no proved drug or treatment available currently. Some studies have shown that vitiligo is correlated to autoimmune problem. Some immune proteins, such as chemokine CXCL10 and receptor CXCR3 were proved to play important roles. In animal, mice with vitiligo demonstrated higher levels of CXCL10 in their skin and blood. Importantly, mice with vitiligo were treated with the neutralizing antibody against CXCL10 can make the mice repigmentation and reduce symptoms of vitiligo. In addition, $CXCR3^{-/-}$ deficient mice treated with CXCR3 antibodies shown reduced symptoms. These data highly suggested that antibodies against CXCL10 or CXCR3 have potential value clinically.

The purpose of this study is to use LAE (Linear Array Epitope) technology to produce repetitive antigen-relative DNA fragments corresponding to the specific amino acid sequence of CXCL10 and CXCR3. The correct DNA fragment is cloned to a protein expressing plasmid and transformed into bacteria for expression of desired protein (named LAE antigen). After expression and purification, the antigens are used to immunize the New Zealand rabbits. The harvested serum (monoclone-like polyclonal antibodies) will be characterized by molecular biology and biochemical tests. These antibodies not only will be further used for academic research, but also will be used to evaluate the feasibility of clinical treatment.

Currently, I have successfully obtained the repetitive target DNA fragments of CXCL10 and CXCR3 through LAE technology, and then connect the protein expression plasmid and send it to bacteria to obtain the DNA sequence of the LAE antigen. The LAE antigen is currently being sent for sequencing confirmation. Next, the antigen will be expressed and purified in bacteria, and the serum will be collected by immunizing New Zealand rabbits. The biochemical properties such as specificity

and sensitivity of the antibody will be evaluated and purified for subsequent related research.