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• 計畫中文名稱	自體免疫病人體內含有不尋常氨基酸的免疫球蛋白的研究		
• 計畫英文名稱	Identification and Characterization of IgG with Unconventional Amino Acid Sequences in Patients with Autoimmune Diseases		
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• 研究人員	楊沂淵 Yang, Yi-Yuan		
• 中文關鍵字	類風溼性關節炎；免疫球蛋白基因；噬菌體呈現系統；胺基酸序列		
• 英文關鍵字	Rheumatoid arthritis (RA)；Immunoglobulin gene；Phage displaying system；Amino acid sequence		
• 中文摘要	查無中文摘要		
• 英文摘要	Our analysis of IgG rheumatoid factors (RFs) from patients with rheumatoid arthritis (RA) revealed that most contained significant numbers of skewed mutation per V region, suggesting that these RFs arose from antigen-driven response. To further study IgG RFs in RA, we used pComb3 vector to construct an IgG1, 1 combinatorial antibody library from a synovial fluid sample. Sequence analysis of 20 randomly chosen Fc-binders demonstrated that all the putative germline V genes for these RFs also encode RF in RA patients. However, none of these RF V regions are similar to those of the two RFs derived by the hybridoma technique from the same synovial sample. Surprisingly, the Humha311 H chain contains 2 frameshifts, resulting in a mainly unconventional amino acid sequence in the CH1 region. To delineate the significance of this unusual CH1 amino acid sequences, we investigated the presence of IgG molecules with such amino acid sequences in the sera of RA patients. As proposed in our study, we have constructed 2 additional combinatorial antibody libraries from the peripheral mononuclear cells of RA patients. However, after extensive effort of screening, we did not identify any IgG Fc-binder containing the unconventional amino acid sequences in the heavy chain gene. The discrepancy in V gene usage among library-derived IgG RFs may reflect: 1) that the sample size is too small; and 2) that the VH and/or the VL region DNA sequences are not expressed as efficiently as the previously isolated ML11 in the other 2 libraries. The results implied that IgG RFs may not be produced in all the RA patients. To investigate the prevalence of the ML11-like protein in the sera of RA patients, we intended to generate anti-idiotypic antibodies against ML11 Fab fragment, which were later used as coating antigens in an ELISA. The Fab fragment of rheumatoid factor		

of ML11 containing unconventional amino acid sequences was purified using anti-human .lambda. antibody affinity column and was subjected to polyacrylamide gel electrophoresis to scrutinize its purity. The purified Fab was visualized with Coomassie blue staining and shown as a single band with a molecular weight of approximately 50Kd under the non-reducing condition (Figure 1). A protein band of 25Kd in molecular weight was detected when the sample loading buffer contains the reducing agent, 2-mercaptomethanol (data not shown). The nature of this protein was further verified by western blot analysis. As shown in Figure 2, two proteins with 25 and 50Kd were detected by anti-human .lambda. light antibody respectively under reducing and non-reducing conditions, suggesting that ML11 Fab was constituted as a heterodimer of heavy and light chains through di-sulfate bonding. This Fab preparation was used to immunize the rabbit to produce polyclonal antibodies, which reactivity was determined by ELISA on a ML11 Fab-coated plate (Figure 3). The binding specificity of the produced anti-ML11 antibodies was analyzed comparatively for their binding to either ML11 Fab molecule or a panel of un-related antigens (type IV collagen, bovine serum albumin, chicken ovalbumin, keyhole limpet hemocyanin, tetanus toxoid and single-stranded DNA). The results showed that the generated polyclonal antibodies specifically recognized the immunized ML11 Fab, but not the other antigens as demonstrated in Figure 4. A total of 100 sera samples have been collected and tested for their reactivity to the generated polyclonal antibodies. Unfortunately, none of the screened sera showed significant difference in binding activity between the RA patients and normal individuals (data not shown). Together, the data indicate that previously isolated ML11 heavy chain with an unconventional CH1 amino acid sequence may be unique to that RA patient.