利用噬菌體基因庫技術來研究類風濕性因子

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從分析類風濕性因子(IgG RFs)的實驗得知，在大部份的IgG RFs的變異區域(variable region)都有為數不少的突變(mutation)。這類的突變結果顯示IgG RFs是由抗原刺激所造成的反應。為了進一步研究IgG RFs在類風濕性關節炎病人(RA patient)身上的致病機轉，我們利用pComb3載體，從一個RA病人的關節液中建立了IgG1，λ免疫球蛋白的聯合抗體基因庫(combinatorial antibody library)經過一連串的篩選，其中71clones的免疫球蛋白的Fab部份可以和Fc分子結合，再分析其中任意挑選的兩個clones的核酸序列顯示17(85%)clones含有相同的重鏈(H)和輕鏈(L)之變異區(variable region)，分別以Humha311和Humla211來代表。其餘的三個clones，兩個利用相同的Humla211輕鏈，但是具有不同重鏈變異區。這些篩選出來的類風濕性因子，大部分的假想種系變異基因(putative germline V genes)也都和類關節炎病人體內發現的類風濕性因子相同，不過它們當中沒有任何一個含有和以前從同一個病人關節液以融合瘤技術(hybridoma technique)所製造出來的IgG RFs的基因類似。值得一提的是Humha311重鏈有兩個框構轉移突變(frameshifts)：一個是單一核酸插入(insertion)在JH區的上游區(upstream)，另一個是在靠近CH1區的末端有四個核酸的缺失(deletion)，結果形成一種在CH1區罕見的氨基酸序列。將來的實驗將研究這類罕見氨基酸序列是否存在IgG分子中，更進一步分析它們對於IgG分子構造和免疫特性的影響。

Rheumatoid synovial fluid-derived IgG rheumatoid factors isolated by surface displaying phage library technique

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Previous studies from the analysis of IgG rheumatoid factors (RFs) revealed that the majority contained significant numbers of skewed mutations per variable (V) region, suggesting that these RFs arose from antigen-driven responses. To further study IgG RFs in patients with rheumatoid arthritis (RA), we used pComb3 vector to construct an IgG, λ combinatorial antibody library from a synovial fluid sample. After panning against intact human IgG, Fab fragments from 71/96 phage clones bound to Fc-coated wells. Sequence analysis of 20 randomly chosen Fc-binders showed that 17(85%) clones had identical H and L chain V regions, represented by Humha311 and Humla211, respectively. Of the remaining three clones, two had the same Humla211 L chain, but each with a different H chain V region. Most of the putative germline V genes for these RFs also encode disease specific RF in RA patients. However, none of these RF V regions are similar to that of the two IgG RFs derived by the hybridoma technique from the same synovial fluid. The Humha 311 H chain has two frameshifts: a one-base insertion upstream of the Jh region and

a four-base deletion near the end of the CH1 region, resulting in a mainly uncoventional amino acid sequence in the CH1 region. In the further, it will be important to study the presence of IgG molecules with such unconventional CH1 amino acid sequences, and the effects of these amino acid sequences on the structures and immunological properties of the IgG moleculels.