Creation of receptor selective protein antagonist for therapy against autoimmune disease

○Yasuo Yoshioka
(The Center for Advanced Medical Engineering and Informatics, Osaka University)

Tumor necrosis factor-α (TNF) induces inflammatory response predominantly through the TNF receptor-1 (TNFR1). Thus, blocking the binding of TNF to TNFR1 is an important strategy for the treatment of many inflammatory diseases, such as hepatitis and rheumatoid arthritis. However, because of the unavailability of any TNFR1-selective antagonist, the therapeutic efficacy of this strategy still remains to be confirmed. In this study, we identified a TNFR1-selective antagonistic mutant TNF and investigated the anti-inflammatory effects of mutant TNF in inflammatory diseases. To create mutant TNF, a phage library displaying TNF variants with randomized sequences at the receptor-binding site was prepared. The library was subjected to several rounds of panning and we succeeded in obtaining TNFR1-selective antagonistic mutant TNF (R1antTNF). The R1antTNF did not activate TNFR1-mediated responses, although its affinity for the TNFR1 was almost similar to that of the human wild-type TNF (wtTNF). The R1antTNF neutralized the TNFR1-mediated bioactivity of wtTNF without influencing its TNFR2-mediated bioactivity and inhibited hepatic injury in an experimental hepatitis model. Next, to improve the in vivo stability of R1antTNF, we engineered PEG (polyethylene glycol)-modified R1antTNF (PEG-R1antTNF). Using our unique site-specific PEGylation technology, we improved the *in vivo* stability of R1antTNF without a loss of its antagonistic activity. Additionally, the anti-arthritis effect of PEG-R1antTNF was equal or superior to the TNF-blocking agent Etanercept in a mouse model of rheumatoid arthritis. Recently, there has been much concern over the reactivation of viral infection caused by TNF blockade. Therefore, we assessed whether PEG-R1antTNF and Etanercept would show similar effects, and found that, unlike Etanercept, PEG-R1antTNF did not reactivate viral infection. These results indicate that selective inhibition of TNF/TNFR1 could be effective in treating RA and that PEG-R1antTNF could serve as a novel anti-inflammatory drug for this purpose.