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Generation of a high affinity humanized anti-IP-10 monoclonal antibody by protein engineering

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HuAIP12 and HuAIP13 are humanized IgG1/k monoclonal antibodies derived from independently isolated murine antibodies AIP12 and AIP13, respectively, which bind to and neutralize human IP-10. Although HuAIP12 and HuAIP13 share a high degree of homology in their V region amino acid sequences (there are two amino acid differences in VH and four in Vk), analyses using competition ELISA and surface plasmon resonance (Biacore) indicated that the binding affinity of HuAIP12 for human IP-10 is higher than that of HuAIP13. Because the humanized antibodies compete with each other for binding to IP-10, it is likely that their parental murine antibodies were derived from common germline VH and VL genes. Mix-and-match analysis of heavy and light chains between HuAIP12 and HuAIP13 indicated that the HuAIP12 VH region is essential for high affinity binding to human IP-10. The HuAIP12 and HuAIP13 VH regions differ only at position 55 (numbered sequentially from the N-terminus of the mature protein) in CDR2 and at position 104 in CDR3. Therefore, each of these positions in the HuAIP12 VH was replaced with the corresponding residue from the HuAIP13 VH (Thr to Ile at position 55, and Gly to Ala at position 104) to identify which of these amino acids is important for the higher affinity of HuAIP12. The substitution in CDR3 reduced the affinity of the variant for IP-10, indicating the importance of Gly at position 104 in the HuAIP12 VH for high affinity binding to IP-10; however, the substitution in CDR2 unexpectedly increased the affinity of this HuAIP12 variant for IP-10 significantly and improved its ability to block IP-10-mediated chemotaxis. This result indicates that Thr at position 55 in the HuAIP12 VH has a negative impact on the binding affinity of HuAIP12 for IP-10. The characteristics of the higher affinity variant make it an excellent candidate for therapeutic testing in autoimmune and inflammatory diseases, such as ulcerative colitis and Crohn's Disease, in which high levels of IP-10 have been associated with disease pathogenesis.