Poster Presentations

[MS5-P40] Unique structural features of human IgG4 Fc. <u>Anna M Davies</u>,[ab] Theo Rispens,[cd] Pleuni Ooijevaar-de Heer,[cd] Hannah J. Gould,[ab] Roy Jefferis,[e] Rob C. Aalberse,[cd] Brian J. Sutton[ab]

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IgG4 is normally the least abundant of the four IgG subclasses in human serum, but displays unique biological properties. IgG4 binds Fcγ receptors poorly compared with IgG1 [1] and does not activate complement [2]. These non-inflammatory properties make IgG4 an attractive subclass for monoclonal antibody therapeutics [3]. Furthermore, while IgG antibodies all comprise two heavy and light chains, connected by inter-chain disulphide bonds, IgG4 is unique in its ability to exist as a half molecule, composed of one heavy and light chain. IgG4 can undergo a process known as Fab-arm exchange (FAE) where half molecules combine to form bi-specific, functionally monovalent antibodies [4]. Such bi-specific format antibodies are also considered to hold promise as therapeutic agents [5]. We report crystal structures of human IgG4 Fc, both recombinant (2.00Å) and serum-derived (2.65Å), revealing unique structural features of IgG4 that may explain its biological activity. These features were not observed in an earlier, low resolution structure of IgG4 Fc, due to extensive disorder [6]. Arg409 from the C_H3 domain, identified as a key residue controlling FAE [7], was previously shown to weaken the interaction of the C_H3 domain dimer by disrupting a conserved water-mediated hydrogen bond network [8]. In the IgG4 Fc structures, Arg409 displays conformational variability, each conformer affecting the C_H3 dimer interface differently, providing further insight into factors controlling FAE. The structures also reveal a novel conformation for the C_H2 domain FG loop, a region involved in both Fcγ receptor and C1q binding [9-12]. The FG loop is structurally conserved in IgG1, but in IgG4 adopts a different conformation, with residues 325-330 flipped away from the C_H2 domain. Pro329, located on the FG loop, is an important residue for both Fcγ receptor and C1q interactions, and as a result of the novel conformation, moves from the conserved position observed in IgG1 by ~7Å. The altered FG loop conformation disrupts the hydrophobic "proline sandwich" interaction between Pro329 from IgG and two tryptophan residues from the Fcγ receptor [10,11], and with the loss of ~150Å^2 buried surface area is consistent with the reduced affinity of IgG4 for FcγRII and FcγRIII compared with IgG1. In addition to directly affecting the position of Pro329, proposed to occupy a pocket at the interface between two C1q chains [9], the altered FG loop additionally affects the structure of the C_H2 domain BC loop. Asp270, proposed to interact electrostatically with C1q [9], is located on this loop. A conformational change alters the position of Asp270 such that it faces toward the interior of the C_H2 domain, unable to interact with C1q. The novel conformation of the IgG4 FG loop may be attributed to two sequence differences (Ala327Gly and Pro331Ser), both of which introduce greater potential for flexibility. The unique features revealed by the high resolution IgG4 Fc crystal structures highlight the importance of extending structural studies to subclasses other than IgG1, to better understand

IgG's biological functions.

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