MS04.15.04 THERAPEUTIC ANTIBODY STRUCTURES OF RAT AND HUMANIZED (CAMPATH-1) Fab FRAGMENTS Anne C.Bloomer, Graham M. T.Cheetham*, Geoffrey Hale*, Herman Waldmann*, MRC Lab. of Molecular Biology, Hills Rd, Cambridge, CB2 2QH, UK, *Sir William Dunn Sch. of Pathology, S. Parks Rd, Oxford, OX1 3RE, UK.

The Campath-1 family of antibodies are able systematically to lyse human lymphocytes with human complement by targeting the small cell-surface glycoprotein CD52, commonly called the Campath-1 antigen. Three of these antibodies have been used clinically for several years providing therapy for patients with an increasing variety of immunologically mediated diseases. We report here the first X-ray crystallographic structures of a Fab fragment from an original rat monoclonal antibody and its humanized counterpart, into which the six complementarity -determining regions of the rat antibody have been introduced.

Crystal structures for this pair of Fab fragments have been refined at 2.6 Å and 3.25 Å, respectively. Translational pseudosymmetry is observed in the rat Campath-1G crystals ($P2_12_12_1$, Z=4) and also in one crystal form of the humanized Campath-1H antibody ($P6_522$, Z=1 and $P6_522$, Z=3).

The light chain variable domains of adjacent molecules of Campath-1H form a symmetrical dimer with an extended intermolecular beta-sheet. Such VL domain dimers have implications for light chain disease and amyloidosis.

Within the antibody-combining sites, which are dominated by the protrusion of side chains Lys54 and Lys56 from hypervariable loop H2, both charge distribution and overall integrity are highly conserved, but large changes in the position of loop H1 are observed with an altered conformation of loop H2. The major determinants of this change are the VH domain framework residues 74 and 24, whose identity differs between these two antibodies.

Sequence data for two different antibodies, having higher and lower affinity for the same antigen, point to essential interactions with the antigen CD52 - a small glycoprotein with a GPI anchor - and its octapeptide mimotope.

These structures provide both a detailed structural insight into the transplantation of an intact antibody-combining site between a rodent and a human framework and also an increased understanding of the specificity and antigen affinity of this pair of Campath-1 antibodies for CD52. This study forms the structural basis for future modification and design of more effective antibodies to this important cell-surface antigen.

MS04.15.05 THE ANTI-TUMOR ANTIBODY BR96: X-RAY STRUCTURES IN ANTIGEN-BOUND AND IN FREE FORM. S. Sheriff*, C. Chang*, P.D. Jeffrey*, J. Bajorath†. Bristol-Myers Squibb Pharmaceutical Research Institute, *P.O. Box 4000, Princeton, NJ 08543, and †3005 First Avenue, Seattle, WA 98121

Selective delivery of cytotoxic agents to tumors can be accomplished by using immunoconjugates containing monoclonal antibodies with specificity for tumor-associated antigens. The murine monoclonal antibody mBR96 (IgG3, κ) was raised against human breast carcinoma cells. For its potential application in cancer chemotherapy, a chimeric form (cBR96) has been constructed from murine variable domains and human κ and γ1 constant domains. BR96 recognizes the Lewis Y (Le^y) tetrasaccharide, which was used for crystallization as the nonoate methyl ester:

Gal (β 1→4) Nag (β 1→3) O1-$(CH_2)_8$-$COOCH_3$
↑ (α 1→2) ↑ (α 1→3)
Fuc Fuc

The structures of cBR96 Fab′, cBR96 Fab′–Le^y and mBR96 Fab–Le^y have been determined by X-ray crystallography. BR96 binds to Le^y and interacts through complementarity determining regions (CDRs): L1, L3, H1, H2 and H3. In the binding site, a number of aromatic residues interact with Le^y: His L27D, Tyr L32, Phe L96, Tyr H32, Tyr H33, Tyr H35, Tyr H50, and Trp H100A (Kabat numbering). In addition to interactions with aromatic groups, several hydrogen bonds are formed between the antibody and Le^y. BR96 also interacts with the $(CH_2)_8COOCH_3$ suggesting that the antibody may be capable of recognizing a larger carbohydrate antigen.

Comparison of antigen-bound and free forms of BR96 show that VL and VH do not re-orient, but that three CDR loops undergo segmental motion (L3), conformation rearrangement (H2) or both (L1) upon antigen binding. In L1, differences are observed that are greater than 10 Å between Cα positions. However, in contrast to other antibodies the conformation of H3 does not change significantly, despite extensive main chain interactions with Le^y.

MS04.15.06 STRUCTURE OF A HUMAN IgM RHEUMATOID FACTOR COMPLEXED WITH ITS AUTOANTIGEN IgG Fc. Corper, A. L.[1], Sohi, M. K.[1], Jefferis, R.[2], Steinitz, M.[3], Feinstein, A.[4], Beale, D.[4], Taussig, M. J.[4], Sutton, B. J.[1*], [1]The Randall Institute, King's College London, 26-29 Drury Lane, London WC2B SRL, UK, [2]Immunology Dept., Birmingham University Medical School, Birmingham, B15 2TT, UK, [3]The Hebrew University, Hadassah Medical School, Jerusalem, Israel, [4]Lab.of Structural Studies, Immunology Dept., Babraham Institute, Cambridge CB2 4AT, UK

We have determined the crystal structure of a complex between a human IgM rheumatoid factor (RF) and IgG Fc, revealing for the first time the nature of autoantibody recognition of an autoantigen. RFs are found in the sera and synovia of patients with rheumatoid arthritis (RA), and form immune complexes which cause inflammation and tissue damage. We crystallised a complex between the Fab fragment an IgM RF from an RA patient (RF-AN), and human IgG4 Fc, with a stoichiometry of 2:1 Fab:Fc. The crystals are space group C2 (a = 160.3Å, b = 81.9Å, c = 64.2Å, ß =98.3°) and diffract to 3.2Å resolution. The structure was solved by molecular replacement, and refined to a final R_{cryst} of 0.22 (R_{free} = 0.29). The epitope in IgG Fc consists principally of residues in the CH3 domain, with a smaller contribution from CH2, and overlaps with the binding sites for the bacterial proteins A and G. The interaction between the antibody and the epitope differs in topology from that of antibody-antigen or complexes seen to date, using only one edge of the potential combining site surface. The small number of contact residues accounts for the low binding affinity of the Fab-Fc interaction. The light chain contributes only a single contact residue, but this is the result of somatic mutation of the germline gene, and implicates a process of antigen driven selection in the generation of this RF. These results provide a basis for the design of agents with therapeutic applications in RA.