Characterization of the interaction of natural and foreign ligands with complement receptor 2 (CD21)

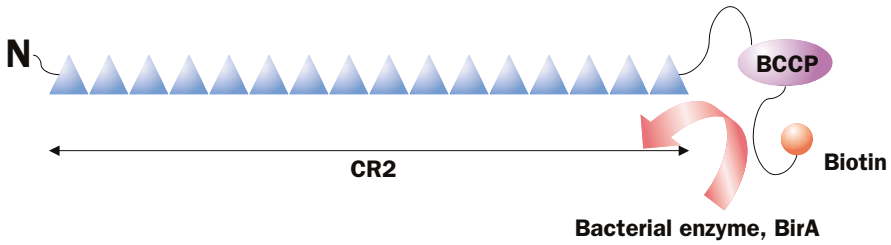

Figure 4A. CR2 was biotinylated at its C-terminus by firstly producing the protein as a construct with the BCCP fragment of *E. coli*-derived acetyl-CoA carboxylase that functions to direct biotinylation at the ligation site on exposure to the enzyme, BirA.

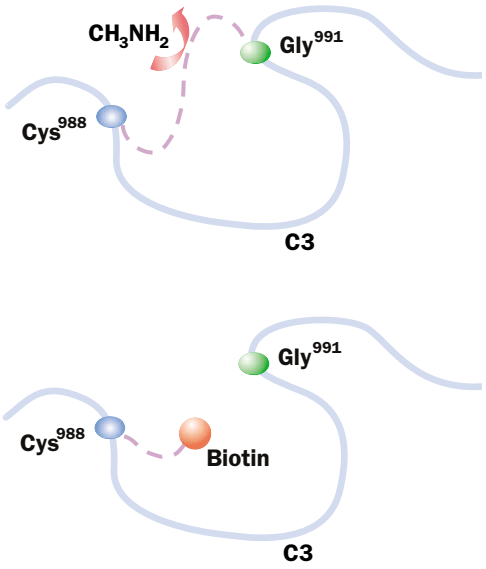

Figure 4B. Biotinylation of of C3 on Cys^{988}. The intra-chain thioester bond between residues Cys^{988} and Gly^{991} was cleaved using methylamine. iC3b and C3d fragments were generated from the same treated stock of biotinylated C3.

Complement receptor 2 (CR2) is a transmembrane glycoprotein expressed on B cells. Ligation with the cleavage products of C3 (iC3b or the subsequent serum protease-derived product, C3d) generated during complement activation is involved in a range of responses e.g. generation of immunological memory, Ig class switching and tolerance. Maria Rosa Sarrias and colleagues at the University of Pennsylvania in Philadelphia used Biacore's SPR technology to differentiate the kinetics of the interaction between C3-derived complement components and CR2 (Sarrias *et al*, 2001). Additionally, the interaction between EBV antigen gp350 (which has been shown to elicit similar responses in B cells as do the "natural" CR2 ligands) and CR2 was characterized.

CR2 or C3-derived components were firstly biotinylated and immobilized on Sensor Chip SA. A key feature of this work was the effort made to ensure that the immobilized ligands were biotinylated in a controlled way to mimic the orientation of CR2 and iC3b/C3d at the cell surface. CR2 was cloned and expressed as a fusion protein with a C-terminal subunit of "biotin carboxyl carrier protein" (BCCP), an active subunit of acetyl-CoA carboxylase from *E. coli*. This subunit contains a lysine residue that acts as a biotin acceptor and thus directs biotinylation to that site. This strategy thus enabled CR2 to be biotinylated on its C-terminal BCCP tag. C3 fragments were biotinylated on the thiol group of Cys^{988}, an amino acid residue that participates in the formation of an intramolecular thioester bond. Following cleavage using methylamine and biotinylation at this site, C3 was then cleaved into its degradation fragments, iC3b and C3d (Figure 4).

A truncated fragment of the EBV surface glycoprotein, gp350 bound to CR2 and the binding data closely fitting a 1:1 model. The calculated affinity dissociation constant (K_D) of 45 nM is some 4-fold lower than that determined when the entire gp350 bound to CR2 in a cell-based assay (Tanner *et al*, 1988), a difference that could indicate that other regions of gp350 influence the interaction.

Kinetic analysis using Biacore suggested that interaction of C3 cleavage fragments with CR2 was more complex and did not fit to a simple 1:1 binding model. The data did however fit a bivalent analyte binding model, which is consistent with data from other studies. Indeed, in addition to the C3d site that was found recently by X-ray crystallography to be involved in CR2 binding (Szakonyi *et al,* 2001), a peptide covering residues 1199-1210 of C3 bound to immobilized CR2 in Biacore experiments. This peptide, which was unable to inhibit binding of C3d to CR2, was characterized by fast dissociation and suggests the presence of a further discrete CR2 binding site on C3d.

References

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