An investigation of the interaction between human complement factor H and C3b.

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The complement system in human blood is a major effector system in humoral/innate immunity (1). Factor H is a 155kD glycoprotein which is involved in the regulation of the complement alternative pathway (2). Factor H regulates C3 turnover by 2 activities: decay acceleration activity (convertase) of the alternative pathway (3). Once factor H is bound to C3b, factor I, a serine protease, cleaves the 11kD alpha chain of C3b to 68 and 43 kD fragments, thus abrogating the action of C3b (4). The latter activity of factor H is termed “Factor I-cofactor activity”. Interaction of factor H with C3b is mainly ionic, and of low affinity, involving several (probably 3-4) weak interaction sites (5).

Structurally, factor H is composed of 20 complement control protein modules (CCPs), each about 60 amino acids long and possessing 4 conserved cysteine residues. 2D proton NMR has revealed that each CCP has a hydrophobic core, with 3 protruding loops of variable sequence, which are likely to form the binding sites for ligands of proteins which contain CCPs (6). Other complement control proteins, DAF, MCP, C4bp and CR1, which have similar activities to factor H, are also composed of CCPs. In CR1, up to 4 contiguous CCPs form a C3b binding site (7). In factor H, previous studies have shown that C3b-binding and cofactor activities are localised to a 38kD fragment containing CCPs 1-5 and part of CCP 6 (8).

In this study a further investigation of the regions within factor H responsible for C3b binding and factor I-cofactor activity was carried out. Assays for factor I-cofactor activity were done as previously described (4). Factor H, factor I and C3b, isolated and prepared from human plasma were used (9, 10). Chemical modifications of factor H were carried out to identify residue types important for factor I cofactor activity (11). Histidine and carboxyl group modifications (by diethyl pyrocarbonate and EDAC, respectively) were found to inhibit completely the cofactor activity. Lysine modifications (by cyanate, acetic anhydride or a succinimide) resulted in only a substantial inhibition whilst arginine modifications (phenylglyoxal) resulted in only minor reduction in the factor I cofactor activity of factor H.

A number of monoclonal antibodies (mabs) against factor H were assessed for their effect on cofactor activity. Several inhibitory mabs, e.g MAH1, MAH3, B2216, MH9 and MH5 could totally inhibit the cofactor activity, suggesting that they bind within the C3b binding site. Mabs OX23 and OX24 have very minor effects on cofactor activity, suggesting that they do not bind within the C3b binding site.

Proteolytic digestions of factor H were carried out to localise the epitopes of these mabs by SDS-PAGE and Western blotting. OX23 binds to a 32kD pepsin fragment, encompassing half of CCP 3, plus CCPs 4-7, and also, consistent with previous findings (8) to the 38kD tryptic fragment composed of CCPs 1-5, suggesting that this region may not be required for the cofactor activity. The inhibitory antibodies MAH1 and MAH3 bind to a 32kD pepsin fragment, encompassing half of CCP 3, plus CCPs 4-7, and also, consistent with previous findings (8) to the 38kD tryptic fragment composed of CCPs 1-5/2.

A direct binding assay for factor H - C3b interaction has been set up using glutaraldehyde-polymerised 125I-labelled C3b which effectively increases the affinity of C3b for factor H. Proteolytic fragments of factor H were transferred to a PVDF membrane and subsequently incubated with 125I-labelled C3b for 1 hr at 37°C, and the blot examined by autoradiography. Results are consistent with those from Western blotting, in that C3b binds to the 32kD pepsin fragment and 38kD tryptic fragment described above. CCPs 4 and 5, and parts of 3 and 6 are common between these fragments, suggesting that these CCPs form a major part of the C3b binding site.

A construct composed of CCPs 3, 4 and 5 of factor H was expressed in the bacterial expression system pGEX-2T (Pharmacia) and partially purified using glutathione-agarose beads. The resulting fusion protein did not exhibit cofactor activity. It did however bind C3b directly on a ligand blot. It also bound OX23, but not OX24 by Western blotting, consistent with previous observations.

These results are consistent with a model for C3b binding to factor H via the loop regions of CCPs 3-5, with a contribution from CCP 2 and/or 6. Discussion of OX24 binding above suggests that CCP 6 may not be involved. The size of the binding region is not inconsistent with biophysical data (12) which measure the length of C3b as 18 nm, equivalent to the length of 4 CCPs. Acknowledgements: We thank A.C. Willis for protein sequencing, Prof. M. Dierich and Drs M. Oppermann and J. Alsenz for supplying some mabs. C.J.S holds an MRC studentship.