Development of a new drug to treat Renal Disease

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Introduction/Aims

- The complement system consists of a group of soluble blood proteins which defend against infection.
- Complement is activated via a long sequence of steps, resulting in the formation of a membrane pore consisting of C5b, C6, C7, C8 and C9n, causing bacterial cell destruction and therefore stopping the infection.
- This system is tightly controlled by a number of proteins. When the function of these proteins is disrupted, disease states occur.
- One example of this is aHUS, where blood vessels and red blood cells are attacked and subsequently kidney failure occurs. Current drugs to treat this condition have significant flaws regarding both cost and side effects. Therefore, an alternative treatment is needed.
- **Aims: I)** To find an alternative drug to treat aHUS using antibodies produced from novel cell lines. **II)** To test the strength of a drug which successfully does this.

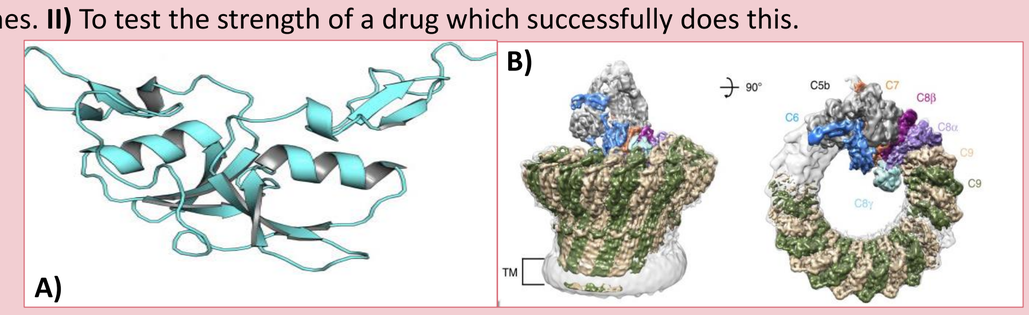

Figure 1 – A) The crystal structure of C7[1]. **B)** Showing the membrane pore formed by the complement system[2]. (TM = transmembrane)

Methods

- Cell lines were grown up and screened for antibody production. These cell lines were tested using an ELISA to determine how well the antibodies were growing. The cell lines were assigned a name according to their position on the ELISA plate for reference.
- These cells were purified using column chromatography (AKTA), then filtered using a P10 drip column. This separated the antibody of interest from any contaminants in the solution.
- These purified antibodies were tested using a haemolysis assay to see how well they block the destruction of red blood cells.
- Finally the binding affinity of 8G5, the most successful antibody, was measured using the Biacore reactor, and a western blot was performed to show this.

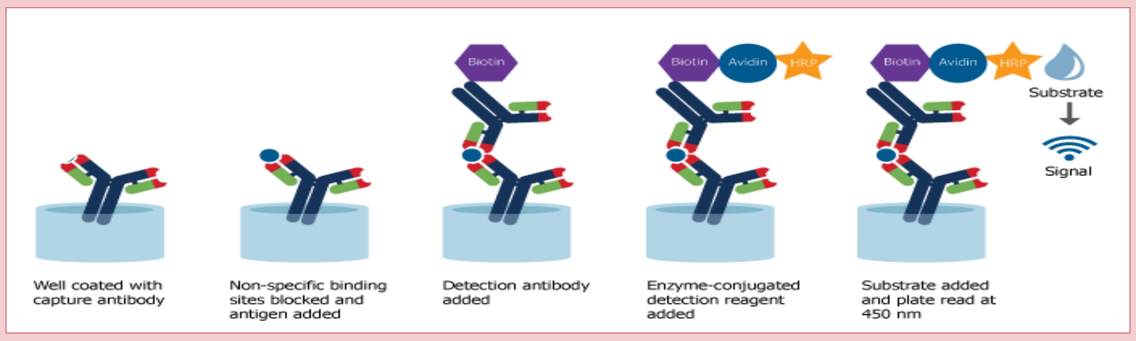

Figure 2 – The technique for the C7 ELISA used to test antibody binding potential [3].

Results

An ELISA was performed on the purified antibodies; it was found that only 8G5 had any C7 binding activity.

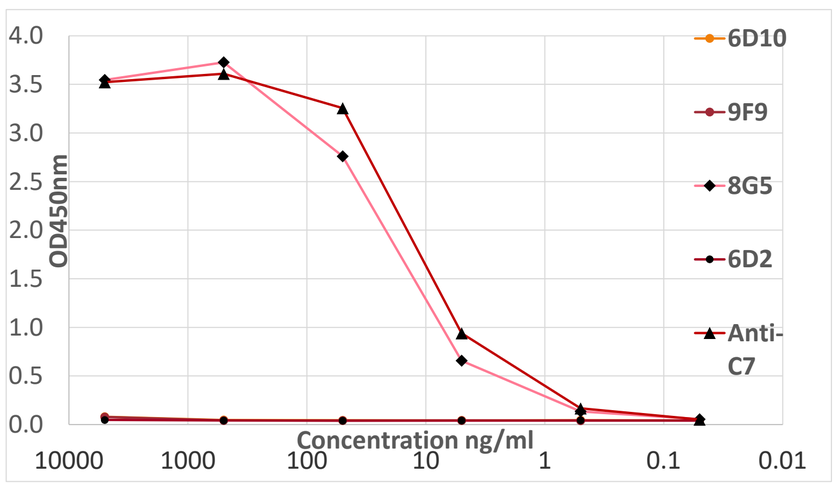

Figure 3 – The results of the first C7 ELISA on the purified antibodies. 8G5 was found to have similar binding potential to the stock anti-C7 antibody (Cardiff University) and so is a potential candidate.

A second ELISA was performed with the non-purified supernatants. This confirmed that all antibodies except 8G5 had lost their binding potential.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.096	0.057	0.039	0.208	0.195	0.189	0.102	0.098	0.099	3.12	2.641	3.135	450
B	0.049	0.044	0.049	0.061	0.061	0.063	0.049	0.062	0.064	0.433	0.418	0.437	450
C	0.048	0.048	0.048	0.295	0.304	0.286	0.136	0.114	0.123	0.059	0.064	0.058	450
D	0.049	0.043	0.048	0.199	0.198	0.194	0.123	0.119	0.132	0.051	0.058	0.05	450
E	0.057	0.048	0.053	0.059	0.06	0.053	0.05	0.046	0.053	0.05	0.054	0.05	450
F	3.292	3.431	3.384	0.048	0.049	0.045	0.045	0.048	0.05	0.052	0.057	0.051	450
G	0.044	0.042	0.045	0.05	0.051	0.048	0.05	0.046	0.053	0.05	0.056	0.055	450
H	0.412	0.056	0.046	0.064	0.05	0.046	0.047	0.057	0.057	0.048	0.062	0.058	450

F1-3 = 8G5; Red = Good binding, white = bad

Figure 4 – Antibodies in triplicate across rows A-H. Row F (8G5) is the only one showing any binding potential.

A haemolysis assay was then performed on the different antibodies to test their pore formation blocking ability. This confirmed that 8G5 had complement blocking activity, however probably not enough to act as a good drug.

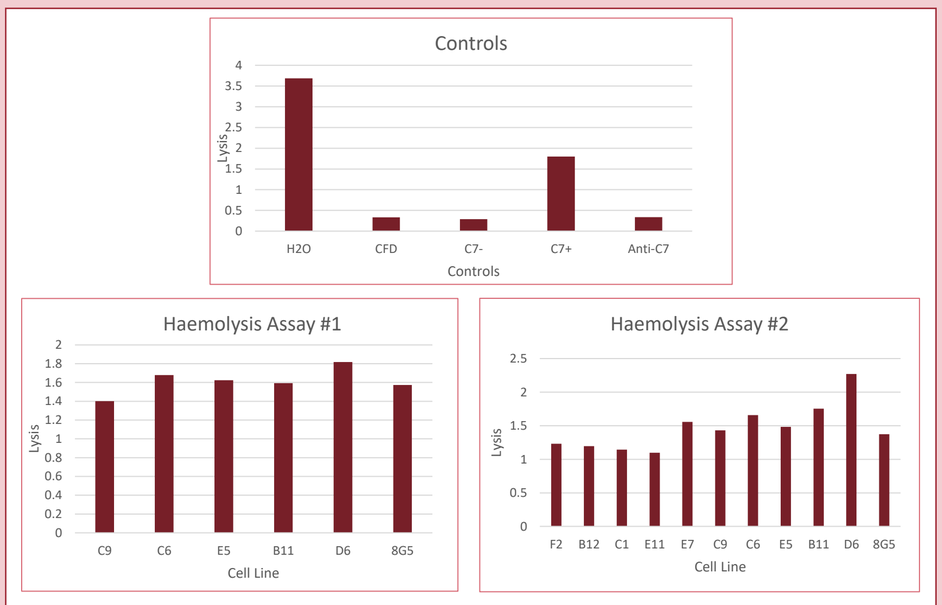

Figure 5 - Two haemolysis assays showing that 8G5 has some complement blocking potential. Interestingly, antibody D6 showed higher lysis than the others; almost as high as the water control, meaning it could potentially act as a complement therapeutic.

Figure 6 – A western blot showing that the 8G5 antibody of interest has the expected molecular weight, and that it binds to C7.
Lane A = Sample
Lane B = Marker

Finally, a western blot was performed. It is clear that a band is visible at around 84kDa which confirms the successful binding of the 8G5 antibody to C7. A Biacore analysis was also performed on this protein to determine the affinity of the interaction, however due to time limitations achieving a clear result was not possible within the scope of this project.

Conclusions

- 8G5 is an antibody which has some C7 blocking activity. It is likely that this can be used in the lab as a diagnostic or research tool despite not being potent enough to act as a drug for clinical use.
- D6 showed increased complement activity – while this is opposite to the effect of the drug we expected to find, this could potentially be looked into further. There is a market for complement enhancing drugs in the treatment of cancers.
- There are more cell lines to be tested – it was not possible to test them all in the time that was given.

References/Acknowledgements

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