Complement Factor H Protein Adhesion on Immune Cells
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Abstract
There were 20,210 deaths due to lymphoma in the US alone in 2010 (Charbonneau et al., 2012), with most patients being treated with rituximab, an antibody treatment that selectively binds to CD-20 expressing B-cells, and is believed to enhance the immune actions of the host (Colombat et al., 2001). However, patients often experience relapses and are refractory to this therapy. Statistical studies have linked underperforming patients to the germline deletion of CFHR1 and CFHR3, proteins affecting the complement system. The complement system is a crucial adaptable chain of proteins whose function is to recognize and eliminate foreign particles. CFHR1 is a family of proteins who are thought to negatively regulate complement activation, although they are largely understudied (Skerra, Chen, Fremaux-Bacchi & Roumenina, 2013). In an attempt to explain why patients who do not express CFHR1 and 3 do not respond well to Rituximab, we must first investigate how CFHR's work by understanding whether they bind to immune cells and if so, where. Previous research on neutrophils has suggested that CFHR1 binds to complement receptor 3 (Loose, Zipfel R, Josii, 2010). Therefore, the experiment blocks for complement receptor 3 as we have no other suggested binding sites. Our main conclusions from our in-vitro assays of Raji cells (B-cell line) and CD8+ and CD4+ T-cells show that we only see some CFHR1 binding on T-cells, and almost none on Raji cells. However, we find that this binding is non-specific, and results do not definitively explain why this is. More experimentation is needed to move forwards.

Methods
1. Cells (Raji B-cells, CD4+ and CD8+ T-cells) extracted and counted
2. FcR block reagent added
3. Select samples blocked with antibodies (anti-CD11b, anti-CD8, IgG2)
4. Human CFHR1+, Human CFHR1- sera added at different concentrations
5. Washes
6. Protein binding tested
   A. Western Blot
   B. Flow Cytometry

Results

Conclusions
◆ Western Blot
a) Raji Cells bind little CFHR1, and this is not CR3 dependent
b) CD4+ and CD8+ T-cells associate more strongly with CFHR1, though this is not CR3 dependent either.

◆ Flow Cytometry
a) Raji Cells bind very little CFH
b) CD8+ T-cells bind some CFH, this is not CR3-dependent.

◆ Implications
a) T-cells are important in anti-tumor immunity
b) Calls for a different approach since association is not through CR3

◆ Future Research
a) Replication of 2010 experiment on neutrophils
b) Repeating western blots with more stringent washes, other antibodies
c) Possible expansion to the other immune cell types

References


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