Progress Report

'Understanding and counteracting antibody-mediated inflammation driving lung damage'.

BACKGROUND:

Chronic bacterial infection of the lung in people with cystic fibrosis (pwCF), especially with species such as *Pseudomonas aeruginosa*, has long been associated with declining lung health and new treatments are urgently needed. Antibodies usually protect us against these bacterial infections, however the T-Wells group discovered 'cloaking antibodies' that paradoxically protect the *Pseudomonas* from killing by our own immune system. Using this information we have successfully treated multiple patients with multi-drug resistant *Pseudomonas* by removing these 'cloaking antibodies' and restoring our own immune systems ability to kill the bacteria.

Despite this success, plasmapheresis is a laborious treatment that is not scalable. Additionally, lung damage in these patients is driven by inflammation, and the link between cloaking antibodies and inflammation is not yet understood.

This project was to identify how these antibodies are linked to inflammation to hopefully identify new treatment options.

PROGRESS:

We have made excellent progress in this project identifying how the antibodies are increasing inflammation in the lung. This falls under two main findings- inflammation produced by macrophages (immune cells that eat bacteria) and inflammation driven by the 'complement cascade' .

Macrophage inflammation:

The main objective was to determine if cloaking antibodies increase inflammatory responses from human macrophages. We have successfully optimised experiments in two types of these human bacteria-eating cells. We found that bacteria coated in cloaking antibodies did increase inflammation when incubated with these macrophages. Inflammation markers IL-6 and TNFa increased rapidly within four hours. However, although these antibodies did increase inflammation, it was not significantly higher than when we used antibodies from other pwCF.

Inflammation from complement:

Early on in this project, we investigated a large data set that suggested cloaking antibodies may be driving production of a very pro-inflammatory protein called 'C5a'. c5a is created when antibodies activate the 'complement cascade'. High levels of C5a in the lungs of pwCF is associated with worse outcomes. To verify this we set up assays to determine if these antibodies made more C5a in the lab, as well as determine if this

antibody led to higher activation of the complement cascade. Our results clearly show that serum from pwCF who have cloaking antibodies activate the complement cascade to much higher rates than pwCF without cloaking antibodies (Fig 1A). These antibodies also generate more c5a in lab assays (Fig 1B).

This is an exciting result as novel therapeutics are already in development that target C5a.

In the last few months of this project, we will finalise both the macrophage data and also strengthen the complement data. We have investigated 10 samples, but will increase this to 40 by the end of the project.

SUMMARY:

1. Our new evidence shows that cloaking antibodies may increase inflammation by over-production of pro-inflammatory C5a.
2. Novel therapies are being developed targeting C5a, pointing the way forward for new treatment methods for pwCF and cloaking antibodies.

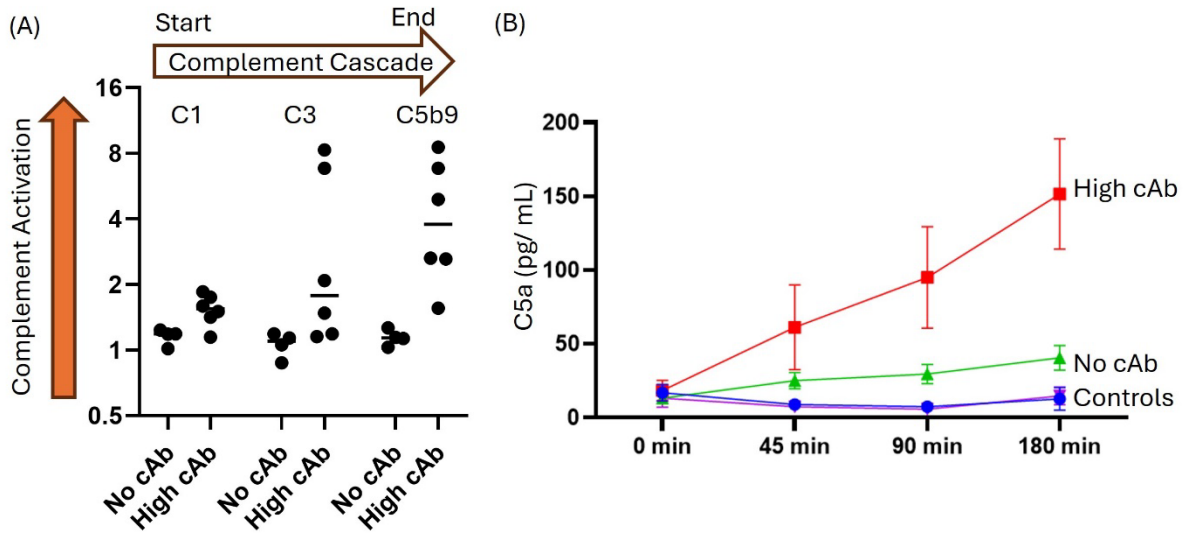

Figure 1. (A) *Pseudomonas* covered in serum with either no cAbs or high cAbs were tested to determine how they activate different steps of the complement cascade. cAbs activated all steps of the cascade higher than serum without cAbs. (B) Serum bactericidal assays show higher generation of C5a if patient serum contains cAb.

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