Antibody-dependent complement deposition (ADCD)- a method optimization

Antibodies can mediate effector functions that can contribute to protection against infectious diseases, one of these functions is antibody-dependent complement deposition[1,2]. In order to evaluate the level of induction of effector functions and its importance in vaccine trials, reliable assays are needed. Therefore, one of my projects focused on optimizing a reproducible, high-throughput assay to measure ADCD in different sample types against various disease antigens.

Being able to measure antibody-mediated complement system activation and deposition of C3 on the surface of infected cells in crucial because the complement system is one of the first barriers of the innate immune system against pathogens. Therefore, ADCD represents an important and valuable effector function against bacteria or viruses. This crucial part of the immune system bridges innate and adaptive immunity and facilitates antibody-dependent killing of pathogens[2]. The complement system consists of a tightly regulated network of soluble proteins in the blood and membrane-associated proteins in tissues. Complement proteins can bind to a variety of cell-bound receptors which activate an enzymatic cascade that aids in host defense by facilitating opsonization of pathogens and their removal via phagocytosis[3,4]. Moreover complement can play a role in the regulation of the adaptive immune response, is involved in tissue regeneration as well as the development of the central nervous system, angiogenic network and embryo implantation[5]. Complement dysregulation on the other hand can result in tissue damage and ongoing inflammatory processes[6].

The complement pathway can be activated via three ways: the classical, the lectin and the alternative pathway, whereas the classical pathway is the only one that can be activated by antibody immune complexes. The degree of activation of the complement system is dependent on antibody isotype, subclass, affinity, and glycosylation state[7–9]. IgM is known to have the greatest potential to fix complement, followed by IgG3, IgG1 and then IgG2 and IgG4[10]. Immune complexes formed by binding of IgG or IgM to pathogens are recognized by a multimeric complex including the complement receptor C1q. Binding of C1q immune complexes starts a cascade involving the formation of a C3 convertase[11]. Cleavage of C3 results in an amplification of the complement cascade with anaphylatoxins acting as a potent chemoattractant for immune effector cells, the production of peptide mediators of inflammation and leukocyte attractors. One of the major mechanisms by how complement mediated the killing of infected cells is via the formation of a membrane attack complex (MAC) which forms a pore by inserting itself into the cell membrane which results in cell lysis[3,12].

The crucial role of the complement system in the removal of opsonized microorganisms reveals itself in individuals with complement deficiencies. For instance, a genetic deficiency can lead to recurrent bacterial infections and higher incidence of meningococcal disease[13]. On the other hand, protective features have been shown against influenza and West Nile virus[14,15]. Activation of complement also plays a role in HIV- infections because HIV-specific antibodies have been found to activate antibody-mediated complement virolysis effectively in early stages in HIV infection[16].

While various assays have been developed to measure the complement activity in human plasma, the majority of these assays do not measure antibody-dependent activation of the complement system. Ayoglu et al. established a bead-based antigen array to measure complement in human plasma samples[17], whereas Schramm et al. developed a lateral flow based assay to detect the concentration of iC3b in human plasma[18]. Jeon et al. used an assay to measure complement system activation in eukaryotic cells by detecting the membrane attack complex C5b-9 on cell surfaces via ELISA[19]. Most of these assays do not measure antibody-dependent activation of the complement system. One antibody-dependent assay used CD4-expressing target cells that were either not pulsed or pulsed with antigen and incubated with antibodies, afterwards human plasma in veronal buffer was added and complement deposition was detected by staining for C3b[20]. The limitation of this widely used assay is the restriction of antigens that can be bound to CD4-cell surface, the usage of both pulsed and un-pulsed CD4 cells doubles the number of wells and the usage of human complement from plasma induces donor variability to the assay.

The antibody-dependent complement deposition assay (ADCD) that our lab aimed to optimize is a bead-based assay using lyophilized guinea pig complement. This simple, reproducible and versatile assay is capable of screening large sample cohorts against different diseases and antigens for complement activating antibodies in a plate format.

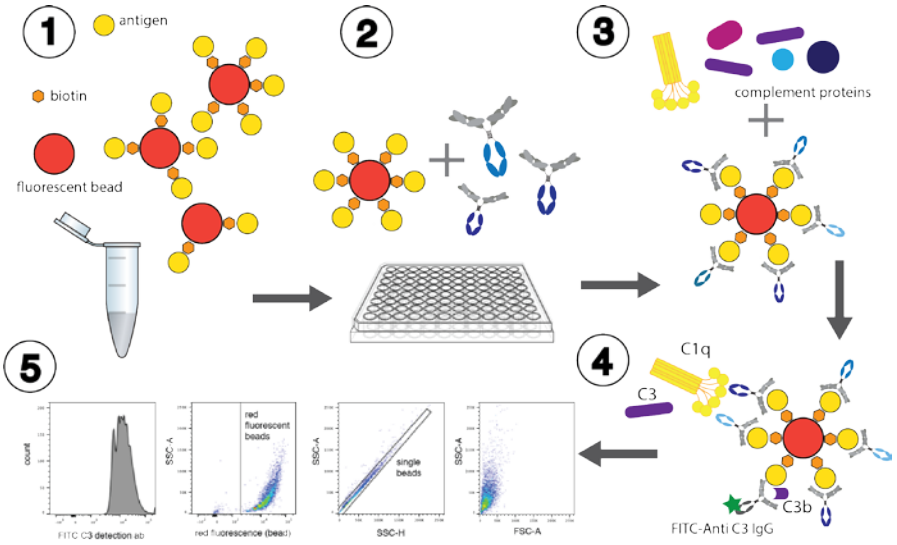

Figure 1: Antibody-dependent complement deposition assay procedure.

Briefly the protocol involves as first step the biotinylation of the antigen of interest and then coupling it to a fluorescent neutravidin bead. The antigen-bound beads are then added to diluted plasma or serum samples in a 96-well plate and incubated at 37C to facilitate the binding of the antibodies in the serum to the antigen. After the incubation, the immune complexes are washed, and guinea pig complement diluted in veronal buffer is added to the complexes. The C1 complex binds to the Fc portion of the antibodies bound to the bead and induces the complement cascade involving C3. The activation of C3 results in the deposition of the opsonizing C3b fragment and the dissociation of C3a. After incubation of complement with the immune complexes at 37C, a detection antibody against the complement C3 molecule is added. The polyclonal antibody detects the C3b that did bind to the bead after cleavage of C3 and soluble C3a was washed away. Afterwards, the samples were washed, and the beads were acquired on a flow cytometer. The events were gated on the single red fluorescent beads and the median fluorescence intensity (MFI) of the secondary antibody is detected as final readout. The main optimization steps

included antigen-bead ratio, sample dilution, immune complex formation incubation time, complement binding incubation time and complement buffer. Antigen-bead ratio did not seem to affect the outcome greatly, whereas longer incubation time allowed the better formation of immune complexes with the best signal to noise ratio at 2h. Incubation time of complement with immune complexes gave the best results for 20min, afterwards, the noise increased rapidly without signal enhancement. Another major change was the buffer in which complement was diluted: the complement system needs calcium and magnesium to be active, in concordance with that fact, veronal buffer with additional calcium and magnesium gave significantly higher results than regular veronal buffer[21]. Lyophilized guinea pig complement facilitates a stable, comparable source of complement which is, in contrast to human complement isolated from blood, not variable between donors. With these adjustments, the assay gives the best signal to noise ratio and is applicable to a variety of disease antigen including HIV, influenza and malaria. Due to the short incubation times and plate format, this assay enabled high throughput work of large sample cohorts.

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