Allosteric inhibition of FcgRIIIa-IgG interactions using Affimers

POS001

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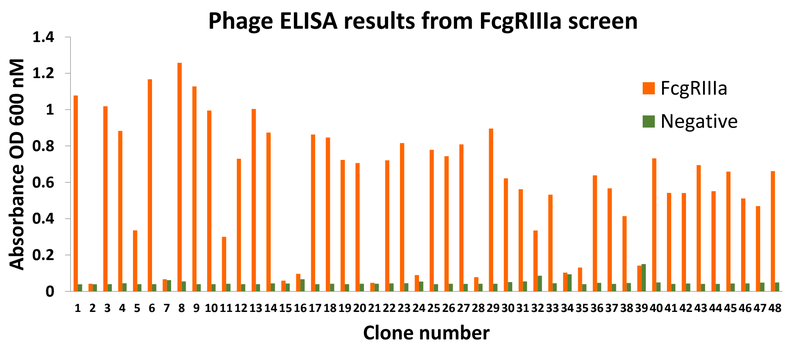
Introduction

The ability to identify allosteric inhibitors of protein function is highly desirable in drug discovery. Here we describe the identification of steric and allosteric binding sites on the human Fcg Receptor IIIa (FcgRIIIa – CD16) using a novel affinity scaffold protein (Affimers). There are two versions of the scaffold, one based on a mammalian Stefin A (Stadler *et al*. 2011) and a second based on plant Cystatin A (Tiede *et al.* 2014). The tertiary structure of both types are homologous and the interaction with the target is mediated via 2 or 3 loops, selected from a highly diverse library using phage display. FcgR-ligand interactions constitute a complex biological system whereby multiple layers of complexity facilitate the fine-tuning of immune responses to infections, whilst maintaining an ability to rapidly switch off these responses following clearance of the infectious/inflammatory stimulus; thus preventing excessive tissue damage.

The screen

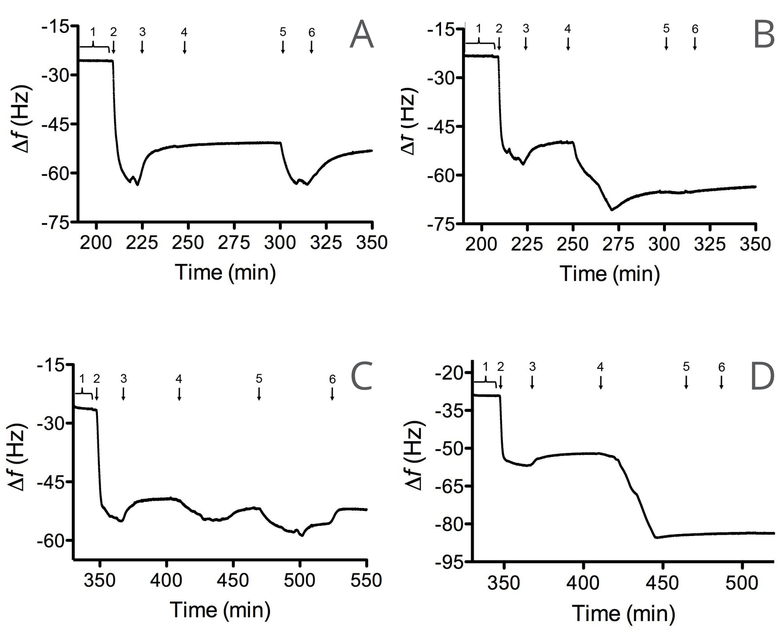
FcgRIIIa was produced by the Oxford Protein Production Facility within the Harwell Research Complex, and biotinylated for phage display using the Affimer libraries. After four panning rounds (taking two weeks), monoclonal reagents were isolated and investigated for binding to FcgRIIa using phage ELISA.



The phage ELISA showed a 80% positive hit rate which isolated six unique Affimer reagents, three were taken forward for further analysis by QCMB, cell based assays and crystallography.

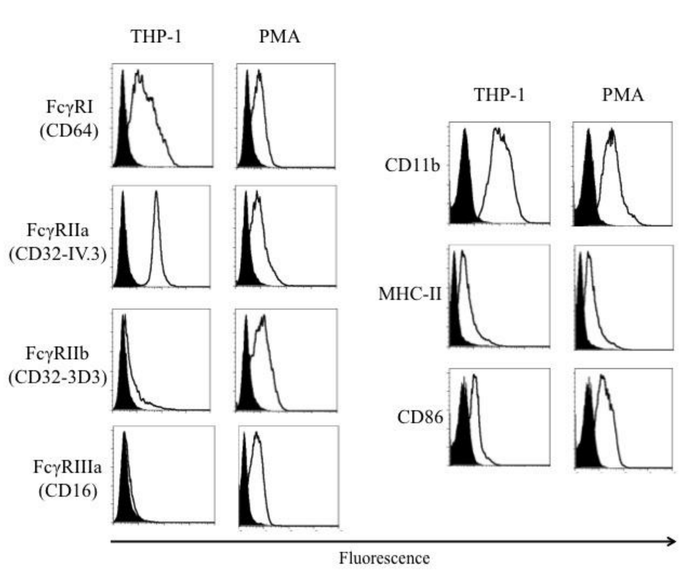
Characterising FcgRIIIa binding Affimers

Quartz Crystal Microbalance was used to determine the ability of the three Affimers to bind to FcgRIIIa and inhibit binding of IgG.



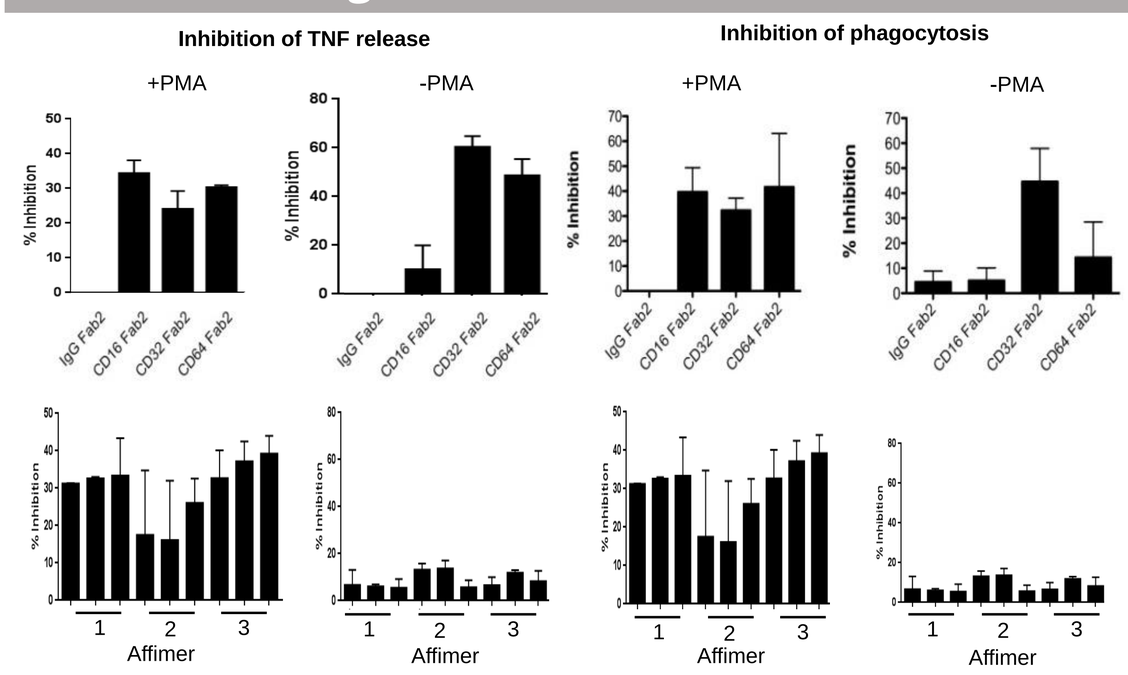
FcgRIIIa was flowed at stage 2 and washed with buffer at stage 3. All Affimers were flowed at stage 4 in Figures B-D. A used a control Affimer. At stage 5 IgGIII was flowed and subsequently washed with buffer 1 at stage 6. All the Affimers tested showed binding to the FcgRIIIa and inhibited IgG binding. Affimers used in B & D showed complete inhibition of IgGIII binding whereas the Affimer in C showed only partial inhibition.

Cell based assays



Flow cytometry examining expression of FcgRs on THP-1 cells with and without PMA induction. FcgRIIIa is only expressed in the presence of PMA in the cell line giving the ability to study specificity and ability to inhibit IgG interaction with the receptor.

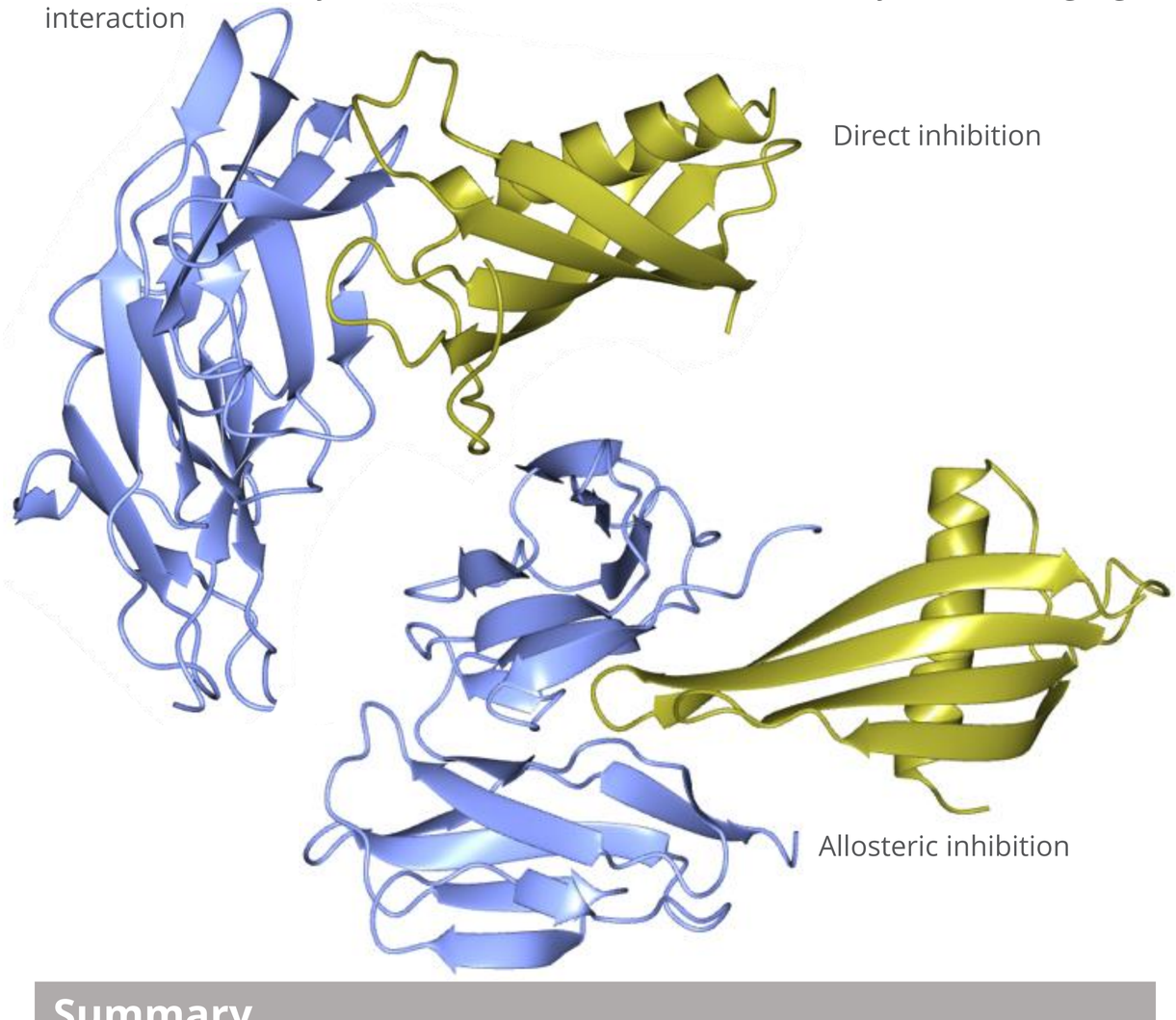
Inhibition of FcgRIIIa activation in cells



All three Affimers showed inhibition of FcgRIII activation in PMA-induced THP-1 cells either via inhibition of TNF release or inhibition of phagocytosis. Controls with FcgR blocking $F(ab')_2$ fragments confirm activity via inhibition of FcgRIII as inhibition with anti-CD16 (FcgRIII) $F(ab')_2$ is seen primarily in PMA-induced cells. CD32 (FcgRII) and CD64 (FcgRI) blocking F(ab')2 fragments inhibit TNF release in non-PMA-induced cells.

Co-crystallisation

The Affimer and FcgRIIIa were co-crystallised at the Oxford Protein Production Facility and solved at Diamond. This revealed two classes of Affimer reagents; Affimers that directly inhibited and Affimers that allosterically inhibited FcgR-IgG interaction



Summary

We have demonstrated the ability to generate Affimers that bind to and inhibit extracellular domains of receptor proteins. These bind with sufficient affinities to completely block ligand binding and show a high level of specificity as they do not bind highly homologous receptor family members. Furthermore owing to their stability and ease of production these have potential as therapeutics in conditions affected by altered FcgR function, such as RA.
Affimers also show promise in identifying allosteric regions of proteins and could be used to guide drug design.

References

Stadler *et al*. (2011) Structure-function studies of an engineered scaffold protein derived from Stefin A. II: Development and applications of the SQT variant. PEDS 24(9) 751-63.
Tiede *et al*. (2014) Adhiron: a stable and versatile peptide display scaffold for molecular recognition applications. PEDS 27(5) 145-155.