Fragment Crystallizable (Fc)-Free Certolizumab Pegol is not Bound by Rheumatoid Factors, while Fc Containing Biological DMARDs Are, Driving Immune Complex Formation and Cellular Clearance

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Objective

To determine why patients with rheumatoid arthritis (RA) and high serum levels of rheumatoid factor (RF) respond differently to various biologic DMARDs.

Background

- RFs are polyclonal autoantibodies which bind the fragment crystallizable (Fc) domain of IgGs.
- Patients with RA and high RF levels have poorer prognosis, more severe progressive disease, greater joint and bone destruction, and decreased response to certain bDMARD therapies versus those with low RF levels.
- Post-hoc analysis of the EXXELERATE phase 4 trial (NCT01500278), showed that in the highest RF quartile patients (>204 IU/mL), adalimumab (ADA) concentrations and treatment target achievement were reduced compared with the lower RF quartiles. By contrast, patients treated with the Fc-free PEGylated Fab, certolizumab pegol (CZP) maintained consistent drug concentrations and had similar clinical outcomes across all RF quartiles.[1]
- In this study, we determine the molecular basis of RF binding to bDMARDS and the potential impact on bDMARD efficacy.

Methods

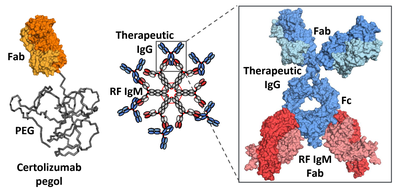
- Monoclonal RF IgMs (RF-AN, RF-61, RF-Yes8cT56K)[2–4] and bDMARDs (ADA, infliximab [IFX], golimumab [GLM], etanercept [ETN], tocilizumab [TCZ], rituximab [RTX], and abatacept [ABT]) were produced in mammalian cells based on published sequences. Certolizumab (CZ) Fab' was produced in E. coli and PEGylated according to manufacturing guidelines.
- Human RA patient sera were obtained from Medix Biochemica.
- RF binding to bDMARDs was assessed by ELISAs and surface plasmon resonance (SPR).
- Therapeutic IgG was modelled from ADA sequence, RF:Fc complex and CZ structures were based on Protein Data Bank: 5WUV, 1ADQ.
- Protein complex formation was assessed by dynamic light scattering (DLS).
- Live cell imaging of primary human macrophages was performed with an Incucyte microscope (Sartorius).

Results

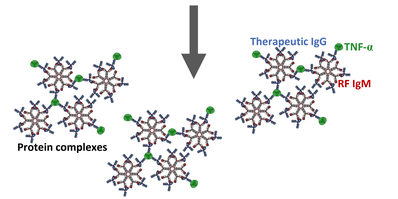
- All 3 monoclonal RF IgMs bound to Fc-containing bDMARDs but were unable to interact with Fc-free CZP by SPR and ELISA (**Figure 1A**).
- RF-Yes8cT56K IgM bound a range of Fc-containing bDMARDs independent of the target antigen of the biologic but not to CZP by SPR and ELISA (**Figure 1B**).
- An IgG Fc domain is required for RF IgM binding by ELISA (**Figure 2A**).
- Binding of Fc-containing biologics by multivalent RF IgMs enabled the formation of large protein complexes as measured by DLS. CZP did not form complexes with RF IgMs (**Figure 2B**).
- The protein complexes were even larger in the presence of TNF-α. CZP bound TNF-α but still did not form complexes with RF IgMs (**Figure 2C**).
- ADA, RF IgM and TNF-α containing protein complexes (green) were bound and cleared by primary macrophages (**Figure 3A**).
- Quantification of the macrophage internalization assays with ADA and CZP was performed (**Figure 3B**).
- Sera from RA patients were designated "low RF" (<70 IU/mL) or "high RF" (>200 IU/mL). Sera with low RF and high RF levels were able to bind ADA by ELISA, but not CZP (**Figure 4A**).
- Sera with low RF levels formed fewer, smaller complexes with ADA compared to serum with high RF levels. High RF sera were able to form large protein complexes with ADA, which were cleared by macrophages (**Figure 4B**).

Summary

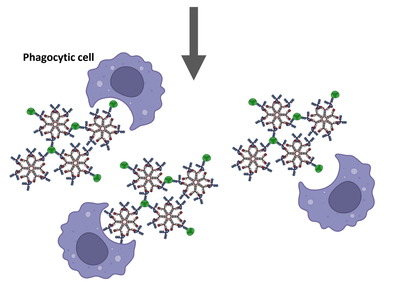
Structural models of Fc-free certolizumab pegol and of RF-AN IgM Fabs in complex with therapeutic IgG



RF IgM binds the Fc domain of bDMARD IgGs

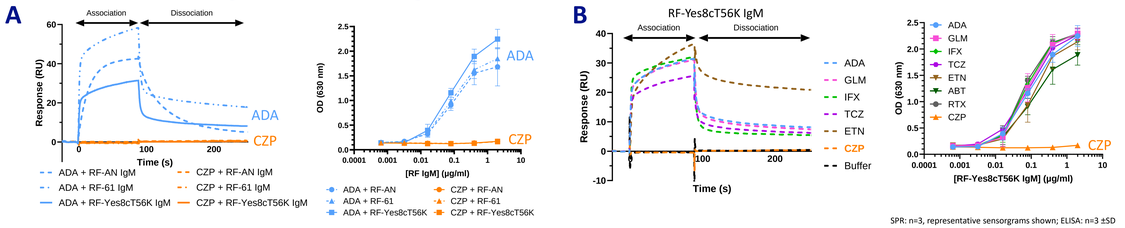


RF IgM forms large protein complexes with bDMARD IgGs and TNF-α



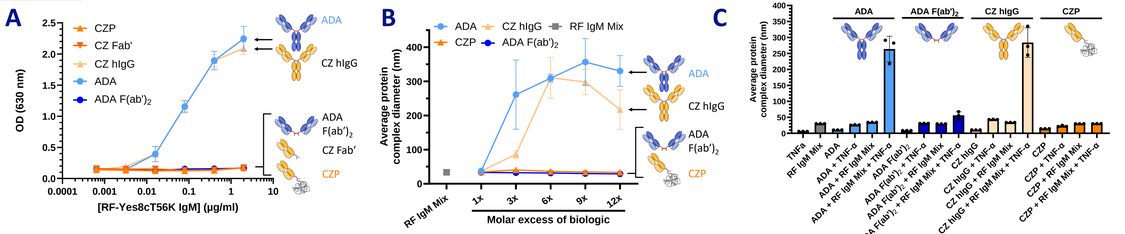
Phagocytic cells clear the protein complexes

Figure 1 Monoclonal RF IgMs bind Fc-containing bDMARDs *in vitro* but not CZP



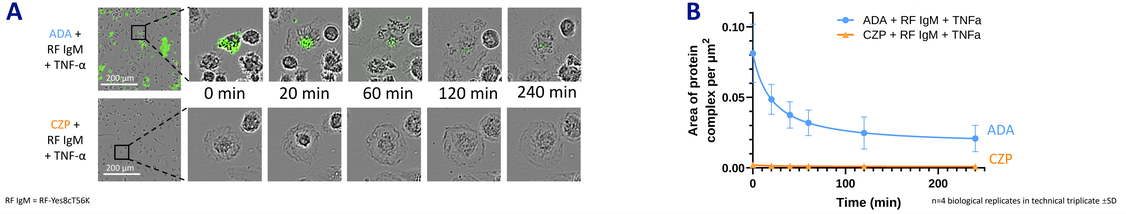
SPR: n=3, representative sensorgrams shown; ELISA: n=3 ±SD

Figure 2 IgG Fc domain is required for RF binding and protein complex formation



n=3 ±SD

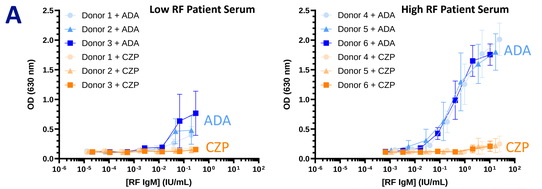
Figure 3 Human macrophages internalize and degrade Fc-containing bDMARD-TNF-α-RF IgM protein complexes *in vitro*



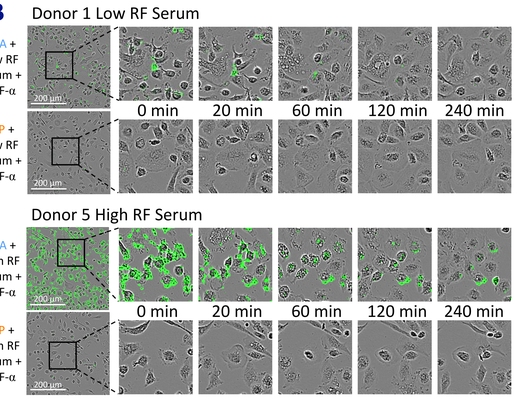
RF IgM = RF-Yes8cT56K

n=4 biological replicates in technical triplicate ±SD

Figure 4 ADA, but not CZP, is bound by RFs in RA patient sera and form protein complexes which are cleared by macrophages



n=3 biological replicates in technical duplicate ±SD



Conclusions

Both monoclonal and patient serum RFs bound to Fc-containing bDMARDs but not to Fc-free CZP. The binding of both monoclonal and patient serum RFs to Fc-containing bDMARDs drove protein complex formation, which stimulated macrophage mediated protein complex clearance. These findings provide insights into why CZP efficacy is independent of patient RF level, while patients with RA and high RF levels exhibit reduced serum drug concentrations and treatment outcomes when treated with Fc-containing bDMARDs, compared to patients with lower RF levels.

ABT: abatacept; **ADA:** adalimumab; **bDMARD:** biologic disease-modifying anti-rheumatic drug; **CZ:** certolizumab; **CZP:** certolizumab pegol; **DLS:** dynamic light scattering; **ELISA:** enzyme-linked immunosorbent assay; **ETN:** etanercept; **Fc:** fragment crystallizable; **GLM:** golimumab; **hIgG:** human immunoglobulin G; **IFX:** infliximab; **IgG:** immunoglobulin G; **IgM:** immunoglobulin M; **IU:** international units; **min:** minutes; **mL:** milliliters; **nm:** nanometers; **RA:** rheumatoid arthritis; **RF:** rheumatoid factor; **RU:** resonance units; **SD:** standard deviation; **SPR:** surface plasmon resonance; **TCZ:** tocilizumab; **TNF-α:** tumor necrosis factor-α; **μg:** micrograms; **RTX:** rituximab.

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References: [1]Smolen J.S. Rheum 2024;00:1–10; [2]Corper A. Nat Struct Biol 1997;4:374–81; [3]Duquerroy S. J Mol Biol. 2007;368:1321–31 [4]Shiroshi M. J Biol Chem. 2018;293:7008–16. **Author Contributions:** Substantial contributions to study conception/design, or acquisition/analysis/interpretation of data: **SRB, SH, KRM, DMK, JO, GO, BL, BU, DH**; Drafting of the publication, or reviewing it critically for important intellectual content: **SRB, SH, KRM, DMK, JO, GO, BL, BU, DH**; Final approval of the publication: **SRB, SH, KRM, DMK, JO, GO, BL, BU, DH**. **Author Disclosures: SRB, SH, KRM, DMK, JO, GO, BL, BU, DH** are employees of UCB; **SRB, DMK, JO, BL, BU, DPH** are stockholders in UCB. **Acknowledgements:** We thank Klaudia Mikula, Jakub Zydron, Hanna Hailu, Ewa Lukijanczuk, Sue Cross, Kerry Tyson for reagent generation, Alison Turner for DLS assay development, Adam Hold and Adam Long for mass spectrometry analysis. Diagrams were designed using BioRender. We thank the patients and their caregivers in addition to the investigators and their teams who contributed to this study. The authors acknowledge Roshni Patel, BSc, Costello Medical, Cambridge, UK for review management and editorial assistance. All costs associated with development of this presentation were funded by UCB.

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