Purified monoclonal rheumatoid factors bind to Fc containing TNF inhibitors *in vitro* but not to the Fc-free TNF inhibitor, certolizumab pegol

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Objectives

To determine the binding properties of rheumatoid factors (RFs) to biological disease-modifying anti-rheumatic drugs (bDMARDs) with or without the fragment crystallizable (Fc) domain.

Background

- RFs are polyclonal autoantibodies which bind to distinct sites on the Fc domain of immunoglobulin Gs (IgGs).[1] RFs are detected in 70–90% of patients with RA, predominantly as immunoglobulin M (IgM) isotype.
- Patients with RA and high RF levels (>200 IU/ml) have poorer prognosis, more severe progressive disease, greater joint and bone destruction and increased cardiovascular disease.[2–4]
- Patients with RA and high RF levels can display a decreased response to bDMARDs.
- Monoclonal RF sequences have been published and enable the expression and purification of monoclonal RF IgMs *in vitro* including RF-AN, RF-61, RF-Yes8cT56K.[5-7]
- A recent post-hoc analysis of the phase 4 EXXELERATE trial (NCT01500278), showed that patients maintained consistent certolizumab pegol (CZP) drug concentrations and treatment target achievement across all RF quartiles, while adalimumab (ADA) concentrations and treatment target achievement was lower in the high RF group compared to patients in the lower RF quartiles.[8]

Results & Conclusions

- The RF IgMs bound to all Fc-containing bDMARDs but were unable to interact with Fc-free CZP. RF IgM binding requires an IgG Fc but is independent of the target protein of the biologic (Figures 1 & 2).
- Binding of ADA by multivalent RF IgMs enabled the formation of large protein complexes. These complexes were even larger in the presence of TNF-α. CZP did not form complexes with RF IgMs (Figure 3).
- ADA, RF IgM and TNF-α containing protein complexes were bound and cleared by primary macrophages (Figure 4).

Impact

These findings provide molecular insights into why patients with RA and high RF levels have different treatment outcomes than those with lower RF levels. This data provides a biochemical explanation as to why ADA drug levels might be lower in high RF than in low RF patients and why CZP drug levels are unaffected by RF levels.

Structural models of RF-AN IgM Fabs in complex with human IgG1 and of certolizumab pegol

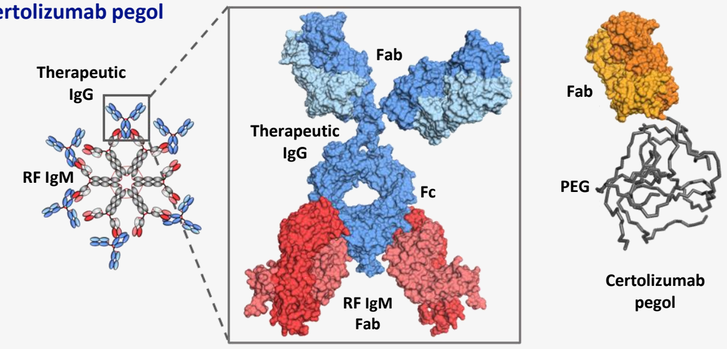
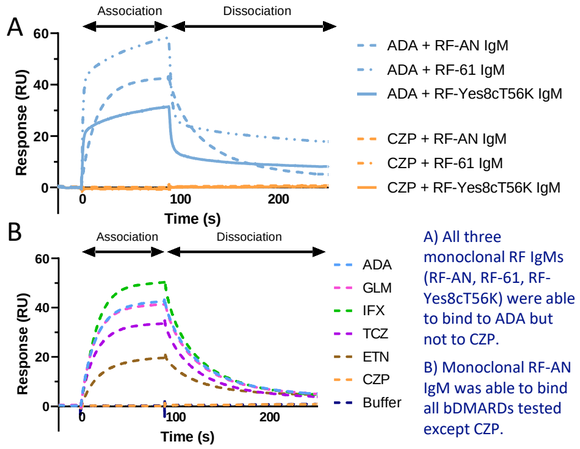


Figure 1 Monoclonal RF IgMs do not bind CZP but do bind to other bDMARDs by surface plasmon resonance (SPR)

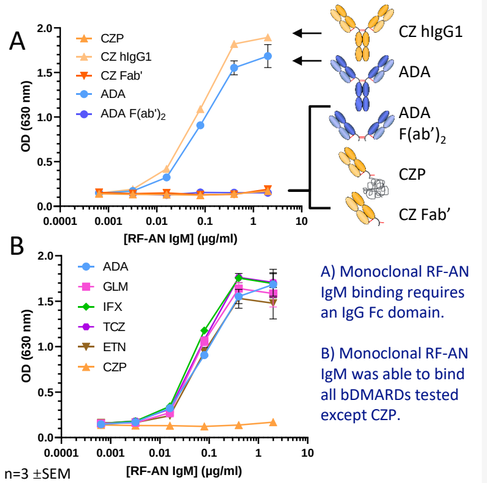


A) All three monoclonal RF IgMs (RF-AN, RF-61, RF-Yes8cT56K) were able to bind to ADA but not to CZP.

B) Monoclonal RF-AN IgM was able to bind all bDMARDs tested except CZP.

n=3, representative sensorgrams shown, RU: resonance units

Figure 2 Monoclonal RF IgMs do not bind CZP but do bind other bDMARDs biologics by ELISA

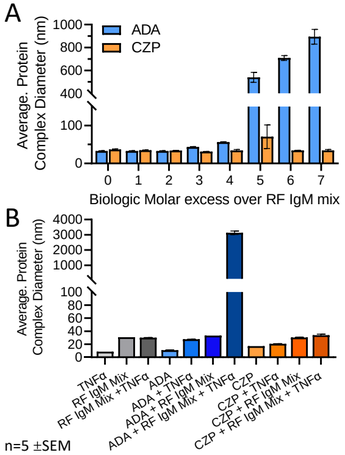


A) Monoclonal RF-AN IgM binding requires an IgG Fc domain.

B) Monoclonal RF-AN IgM was able to bind all bDMARDs tested except CZP.

n=3 ±SEM

Figure 3 ADA but not CZP forms large protein complexes *in vitro* when incubated with mixtures of monoclonal IgM RFs with or without TNF-α



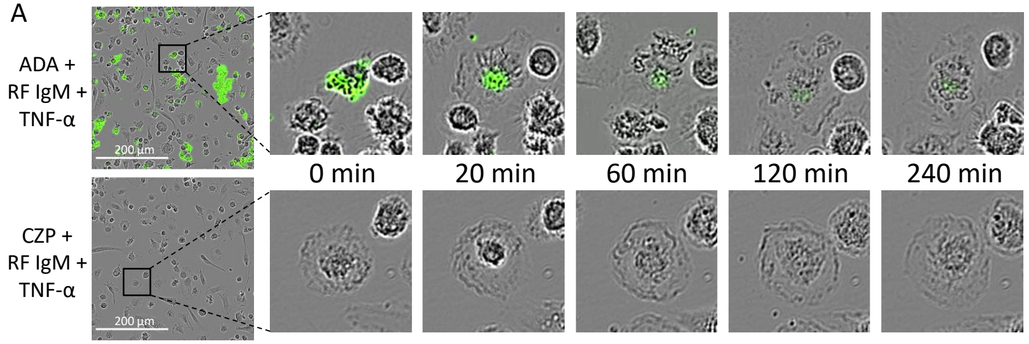
A) A pseudo-polyclonal RF IgM mix (of the 3 monoclonal RF IgMs) formed large protein complexes when incubated with an excess of ADA, as analysed by dynamic light scattering (DLS). The RF IgM mix did not form complexes when incubated with CZP.

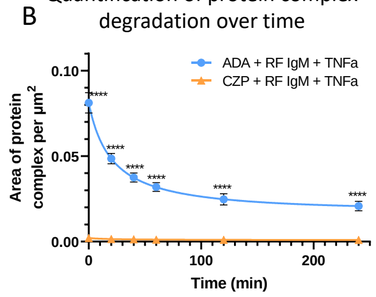
B) Proteins were analysed alone or in 1:1 molar mixtures by DLS. ADA formed even larger protein complexes in the presence of TNF-α and the pseudo-polyclonal RF IgM mix. CZP did not form large protein complexes.

n=5 ±SEM

Abbreviations: ADA: Adalimumab, GLM: Golimumab, IFX: Infliximab, TCZ: Tocilizumab, ETN: Etanercept, CZP: Certolizumab pegol, CZ: Certolizumab

Figure 4 Human macrophages internalise and degrade ADA-TNF-α-RF IgM protein complexes *in vitro*





A) RF-Yes8cT56K IgM, bDMARDs and TNF-α were mixed at the molar ratio 1:9:1 and incubated with primary macrophages at 4°C. Cells were washed and remaining protein complexes detected with Fab anti-human Fab FITC. Cells were moved to 37°C and imaged for 4 hours (Incucyte, Sartorius). ADA formed complexes that were bound and cleared by the macrophages. CZP did not form protein complexes.
B) Quantification of the protein complex area in the images over time, presented as area of protein complex per µm².

n=4 biological replicates in technical triplicate ±SEM, **** P<0.0001 by 2-way ANOVA

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