Rheumatoid Factors (RFs) in RA Patient Sera Do Not Bind To Fc-Free Certolizumab Pegol, But Do Bind To Fc-Containing Anti-TNF-α Biological DMARDs, Driving Immune Complex Formation and Cellular Clearance

0074

Sophie Hopkin,[1] Kathryn R Malpas,[1] David M Kallenberg,[1] Jacqueline O'Neill,[1] Geofrey Odede,[1] Kerry Tyson,[1] Sue Cross,[1] Tatiana Sokolova,[2] Bernard Lauwerys,[3] Baran Ufuktepe,[3] Partick Durez,[2] Susanna R Bidgood,[1] **David P Humphreys[1]**

1. UCB Pharma UK, Slough, U.K., 2.Université Catholique de Louvain, Brussels, Belgium, 3. UCB, Brussels, Belgium

Objective

To determine whether RFs in the sera of patients with RA can bind and promote clearance of biological DMARDs with or without an Fc domain.

Background

- RF seropositivity, detected in the majority of patients with RA, is a key diagnostic marker. High RF levels are associated with poorer prognosis, more severe progressive disease, greater joint and bone destruction and increased cardiovascular disease.[1,2]
- RFs are polyclonal autoantibodies which bind the Fc domain of IgGs.
- Patients with RA and high RF levels experience reduced serum drug concentrations and disease control when treated with Fc-containing biological DMARDs (bDMARD).[3]
- Fc-free certolizumab pegol (CZP) exhibits stable serum drug concentrations and efficacy independent of patient RF levels.[4-7]

Methods

- Direct binding ELISAs: Plates were coated with 2 µg/ml of bDMARDS, challenged with a titration of monoclonal RF-Yes8cT56K IgM or serum from a biologic-naïve patient with RA, detected with HRP-conjugated anti-human IgM and read using a Synergy2 microplate reader (BioTek). Statistical analysis: an unpaired t-test with Welch's correction.
- Microscopy: Sera from biologic-naïve patients with RA, bDMARDs and TNF-α were mixed and incubated with primary human macrophages at 4°C. Cells were washed and bound protein complexes detected with Fab anti-human IgM FITC. Cells were moved to 37°C and imaged for 4 hours (Incucyte, Sartorius). Overlayed phase contrast and green channel representative images are shown. The average area of protein complexes in the images were quantified using Fiji ImageJ. Statistical analysis: Figure 3B, a paired two-tailed t-test on log-transformed data. Figure 4B & 5B, a paired one-way ANOVA with Dunnett's multiple comparison test on log-transformed data (compared to CZP group).

Results

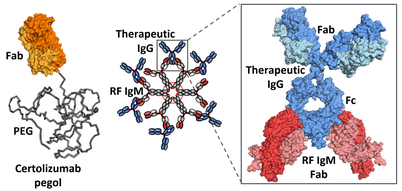
- Monoclonal recombinant RF IgM bound a range of Fc-containing bDMARDs independent of the target antigen of the biologic, but not to CZP (**Figure 1A**).
- An IgG Fc domain is required for RF IgM binding (**Figure 1B**).
- In low and high RF patient sera, RFs bound to Fc-containing ADA by ELISA but not to Fc-free CZP. In RF negative sera, no binding was detected to either bDMARD (**Figure 2A**).
- The magnitude of RF binding to ADA was greater in high RF sera than in the low RF sera. RF negative patient sera had no detectable binding to either bDMARD (**Figure 2B**).
- High RF patient sera formed protein complexes with ADA and TNF-α but not with CZP. No protein complexes were formed in RF negative sera (**Figure 3**).
- An IgG Fc domain is required for protein complex formation in high RF patient sera (**Figure 4**).
- RF-bDMARD-TNF-α protein complexes were rapidly cleared by primary human macrophages (**Figure 5A**).
- High RF patient sera formed protein complexes with Fc-containing ADA, IFX, and GLM in the presence of TNF-α but not with Fc-free CZP. (**Figure 5B**).

Conclusion

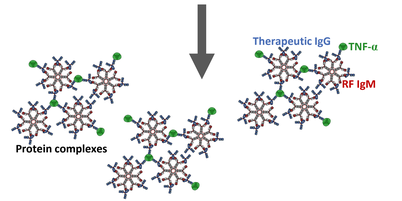
In high RF patient sera, RFs bound Fc-containing bDMARDs (ADA, GLM, IFX) and formed protein complexes, which were rapidly cleared by macrophages. Fc-free CZP was not bound by RFs and was not subject to enhanced clearance.

Summary

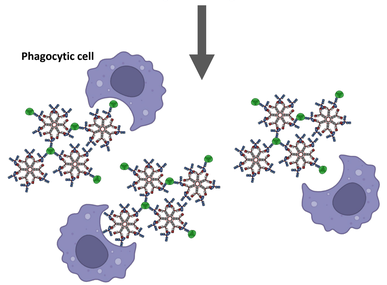
Structural models of Fc-free certolizumab pegol and of RF IgM Fabs in complex with therapeutic IgG



RF IgM binds the Fc domain of bDMARD IgGs

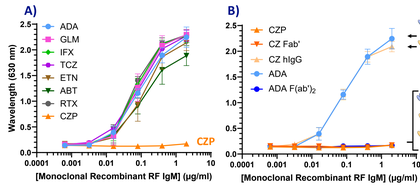


RF IgM forms large protein complexes with Fc-containing bDMARD IgGs and TNF-α



Phagocytic cells clear the protein complexes

Figure 1 Monoclonal RF IgM binds Fc-containing bDMARDs *in vitro* but not Fc-free CZP

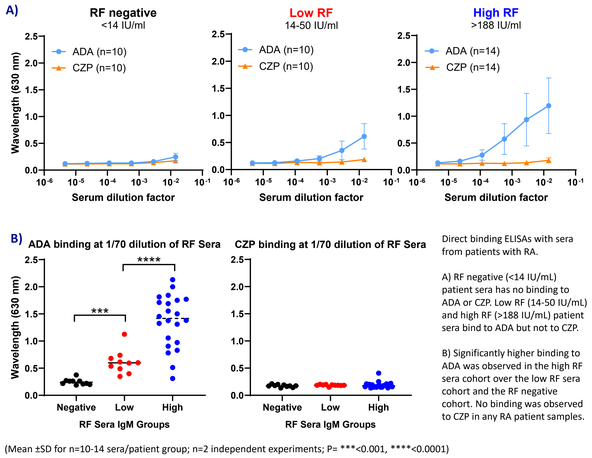


Direct binding ELISAs with monoclonal recombinant RF-Yes8cT56K show that:

A) All Fc-containing bDMARDs were bound by RF but not Fc-free CZP

B) An IgG Fc domain is required for RF binding

Figure 2 RFs in sera from patients with RA bound ADA but not CZP



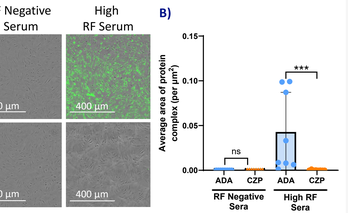
Direct binding ELISAs with sera from patients with RA.

A) RF negative (<14 IU/mL) patient sera has no binding to ADA or CZP. Low RF (14-50 IU/mL) and high RF (>188 IU/mL) patient sera bind to ADA but not to CZP.

B) Significantly higher binding to ADA was observed in the high RF sera cohort over the low RF sera cohort and the RF negative cohort. No binding was observed to CZP in any RA patient samples.

(Mean ±SD for n=10-14 sera/patient group; n=2 independent experiments; P= ***<0.001, ****<0.0001)

Figure 3 RFs in sera from patients with RA form protein complexes with ADA but not with CZP

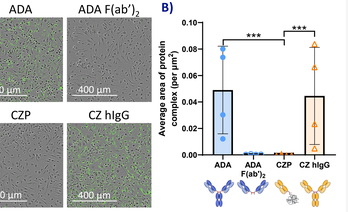


Microscopy images of macrophages incubated with mixtures of sera from patients with RA, ADA or CZP and TNF-α at timepoint 0 min.

A) ADA formed protein complexes in the high RF patient serum, CZP did not. In RF negative serum, no protein complexes were formed with ADA or CZP.

B) The average area of protein complexes in microscopy images for the RF negative and high RF RA patient sera groups. (Each point represents one RA patient serum sample. Mean ±SD for n=8 RA patient sera. P = ***<0.001)

Figure 4 IgG Fc domain is required for protein complex formation in high RF patient sera

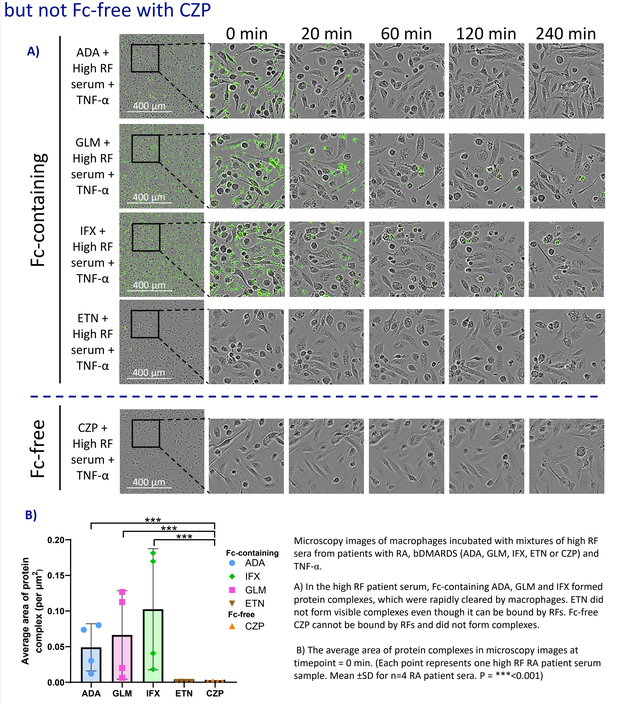


Microscopy images of macrophages incubated with mixtures of high RF sera from patients with RA, ADA or CZP and TNF-α at timepoint 0 min.

A) Fc-containing ADA and CZ hIgG formed protein complexes in the high RF patient serum, Fc-free CZP and ADA F(ab')$_2$ did not.

B) The average area of protein complexes in microscopy images. (Each point represents one RF high RA patient serum sample. Mean ±SD for n=4 RA patient sera. P = ***<0.001)

Figure 5 High RF patient sera form protein complexes, which are rapidly cleared by macrophages, with Fc-containing ADA, GLM and IFX but not Fc-free with CZP



Microscopy images of macrophages incubated with mixtures of high RF sera from patients with RA, bDMARDS (ADA, GLM, IFX, ETN or CZP) and TNF-α.

A) In the high RF patient serum, Fc-containing ADA, GLM and IFX formed protein complexes, which were rapidly cleared by macrophages. ETN did not form visible complexes even though it can be bound by RFs. Fc-free CZP cannot be bound by RFs and did not form complexes.

B) The average area of protein complexes in microscopy images at timepoint = 0 min. (Each point represents one high RF RA patient serum sample. Mean ±SD for n=4 RA patient sera. P = ***<0.001)

Abbreviations: ABT: abatacept; **ADA:** adalimumab; **ADA F(ab')$_2$:** Fc-free ADA Di-Fab; **bDMARD:** biologic disease-modifying anti-rheumatic drug; **CZ Fab':** certolizumab Fab'; **CZ hIgG**: Certolizumab Fab' reformatted as human IgG1; **CZP:** certolizumab pegol; **ELISA:** enzyme-linked immunosorbent assay; **ETN:** etanercept; **Fc:** fragment crystallizable; **GLM:** golimumab; **IFX:** infliximab; **IgG:** immunoglobulin G; **IgM:** immunoglobulin M; **IU:** international units; **min:** minutes; **ml:** milliliters; **nm:** nanometers; **RA:** rheumatoid arthritis; **RF:** rheumatoid factor; **SD:** standard deviation; **TCZ:** tocilizumab; **TNF-α:** tumor necrosis factor-α; **µg:** micrograms; **RTX:** rituximab.

References: [1.] Sobhy N. Egypt Rheumatol. 2022;44(4):325–8; [2.] Fazeli MS. Clin Med Insights Arthritis Musculoskelet Disord. 2021;14:11795441211028751; [3.] López-Medina C. RMD open. 2024;10:e003975; [4.] Martínez-Feito A. Clin Exp Rheumatol. 2024;42(5):999-1005; [5.] Smolen J S. Rheumatol. 2024;63(11):3015-24; [6.] Smolen J S. Arthritis Rheumatol. 2024;76(suppl 9); [7.] Miyazaki Y. Mod Rheumatol. 2025;35:S56. **Author Contributions:** Substantial contributions to study conception/design, or acquisition/analysis/interpretation of data: **SH, KRM, DMK, JO, GO, KT, SC, TS, BL, BU, PD, SRB, DH**; Drafting of the publication, or reviewing it critically for important intellectual content: **SRB, BU, DPH**; Final approval of the publication: **SH, KRM, DMK, JO, GO, KT, SC, TS, BL, BU, PD, SRB, DH**. **Author Disclosures: KRM, DMK, JO, GO, KT, SC, BL, BU, SRB, DPH** are employees of UCB; **DMK, JO, GO, KT, SC, BL, BU, SRB, DPH** are stockholders in UCB. **Acknowledgements:** We thank Klaudia Mikula, Jakub Zydron, Hanna Hailu, Ewa Lukijanczuk for reagent generation, Adam Hold and Adam Long for mass spectrometry analysis. Diagrams were designed using BioRender. Serum samples from patients with RA were kindly provided by **TS & PD**, Université Catholique de Louvain, Brussels, Belgium. We thank the patients and their caregivers in addition to the investigators and their teams who contributed to this study. The authors acknowledge Emily Johnson, BSc, Costello Medical, London, UK for review management and editorial assistance. All costs associated with development of this presentation were funded by UCB.

To receive a copy of this poster, scan the QR code. Link expiration: 27th January 2026