A new strategy to improve complement dependent cytotoxicity of Rituximab in B-lymphocytes with low CD20

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Introduction

Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults in the western world. One of the treatments offered to CLL patients is immunotherapy, such as Rituximab (RTX), a chimeric IgG drug that binds specifically to CD-20, which is expressed on B cells in almost all stages of development. During immunotherapy, the complement system is involved in various mechanisms that destroy the target B-cells. One mechanism is the complement-dependent cytotoxicity (CDC), which requires the activation of the complement classical pathway (CP). This activation of the CP requires formation of RTX-aggregates. The availability of complement components and the level of CD-20 expression on the surface of B-cells affect the immunotherapy outcomes. Alas, the expression of surface CD-20 on B-cells is decreased in treated CLL patients by post-translational mechanisms.

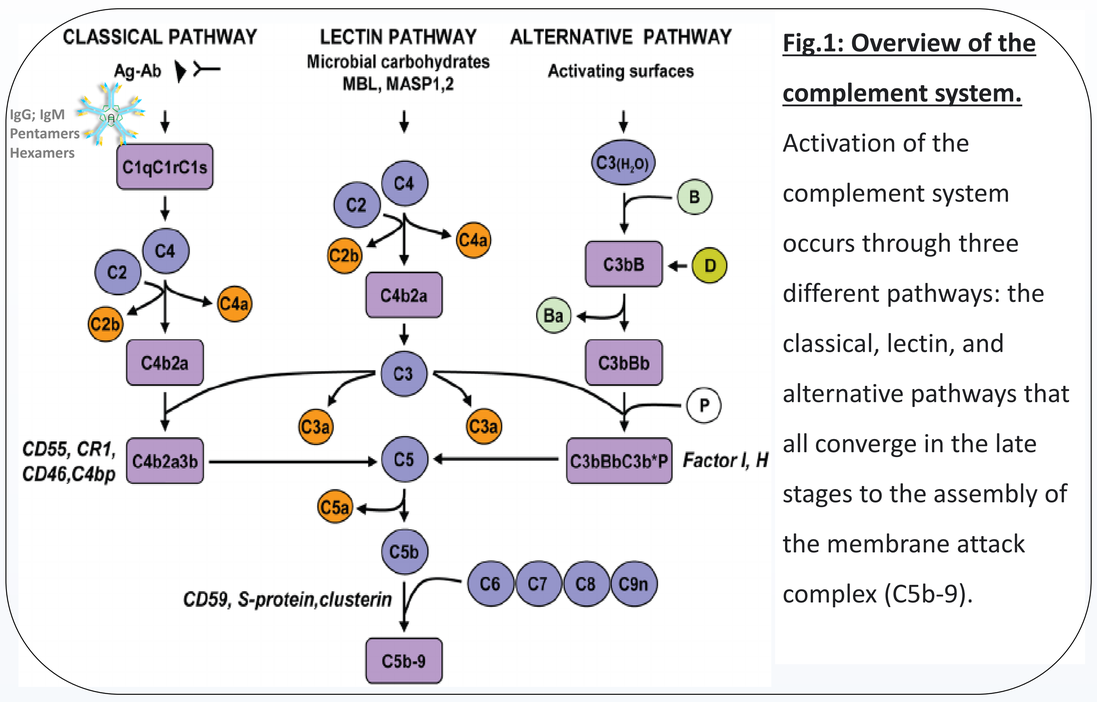

Fig.1: Overview of the complement system. Activation of the complement system occurs through three different pathways: the classical, lectin, and alternative pathways that all converge in the late stages to the assembly of the membrane attack complex (C5b-9).

Hypothesis

We hypothesized that the use of aggregated- instead on monomeric- anti CD-20 antibodies may increase the activation of CP and improve the CDC efficacy in CLL B-cells with low levels of surface CD-20.

Aims of the study

1. To study the potential benefit to CDC of pre-aggregated RTX.
2. To find the optimal method for RTX aggregation, aimed to improve CDC efficacy.

Contribution to science

Understanding and improving the CDC mechanism by aggregated anti-CD-20 drugs may be used in the future to refine and advance personalized therapies in CLL patients that express low level of surface CD-20 on B-cells.

Materials and methods

- Blood samples were collected from naïve CLL patients and normal controls (NC).
- RTX was separated into two fractions: solution (Sol.) and antibody (Ab.).
- RTX, Ab. and Sol. were heat aggregated (H.A) at 63°C for 20 min or aggregated by agitation (Ag.) for 3 days at room temperature.
- Aggregates were detected using Dynamic Light Scattering (DLS).
- The ability of aggregated RTX to activate CP was examined.
- MEC-2 (high CD-20) and HG-3 (low CD-20) cell lines were used for the CDC assay.

Results

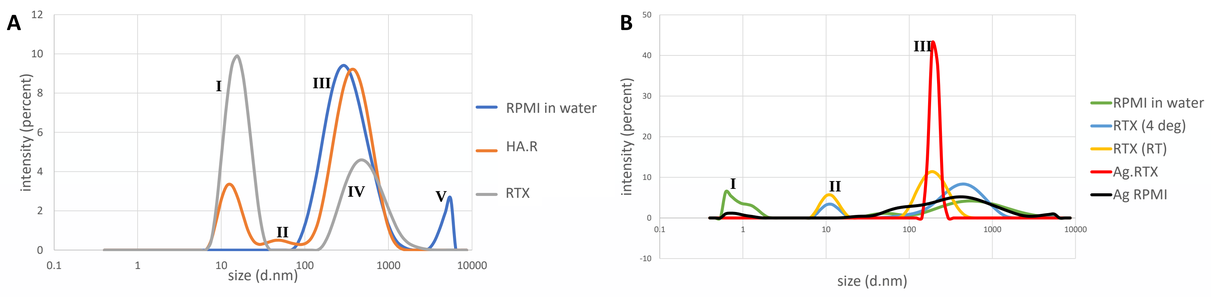

Fig.2. Dynamic light scattering results: DLS results showing five peaks in heat-aggregated samples (A), representing monomeric RTX (I), aggregated RTX (II and IV), and a typical peak present in water samples (III). V may represent larger aggregates. (B) aggregation via agitation showing three peaks: a typical peak in water (I) , monomeric RTX (II), and aggregated RTX (III).

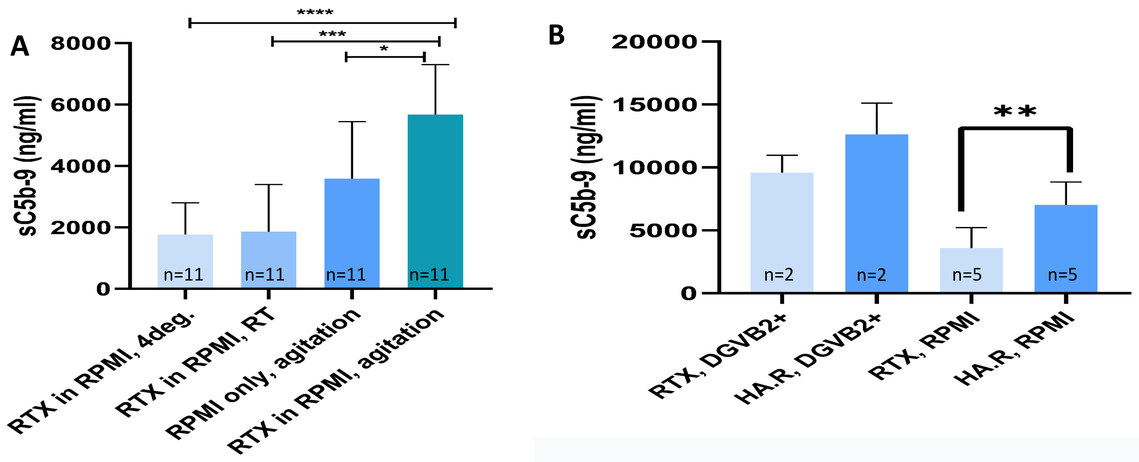

Fig.3. Complement activation by in-vitro aggregated RTX: The results show increased CP activation by the heat aggregated RTX (HA.R) relative to non-aggregated RTX (RTX) in activity buffer ($DGVB^{2+}$) and RPMI medium (A). The increase in CP activation by the agitated RTX relative to non-agitated RTX was shown (B).

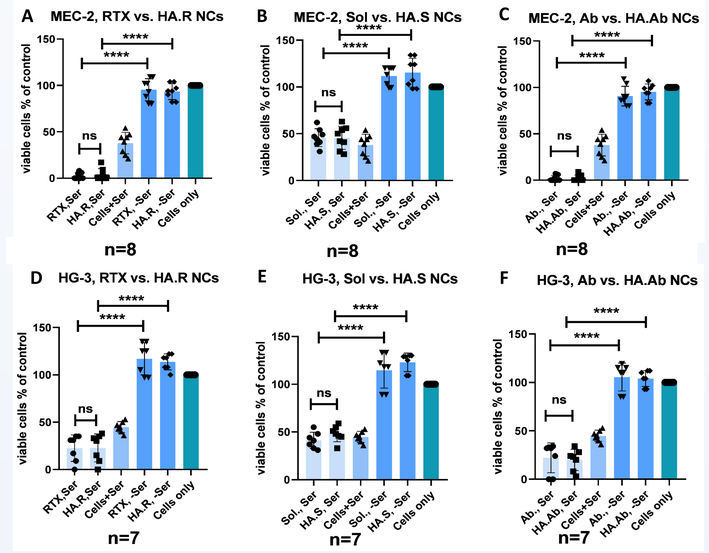

Fig.4. CDC assay in MEC-2 and HG-3 cells. The results show that viability of MEC-2 cells was very low (i.e. CDC was high) after incubation with serum and RTX or HA.R (A), and Ab. or HA.Ab (C). Sol. and HA..S did not induce CDC (B). In HG-3 cells the results didn't show difference in cells viability after incubation with serum and RTX or HA.R (D), Sol. and HA.S (E) and Ab. or HA.Ab (F).

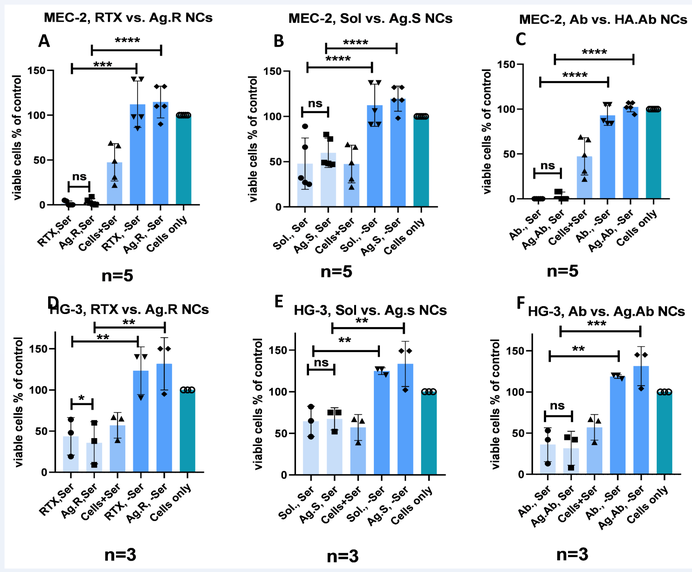

Fig.5. CDC assay in MEC-2 and HG-3 cells. The results show that viability of MEC-2 cells was very low (i.e. CDC was high) after incubation with serum and RTX or Ag.R (A), Ab. or Ag.Ab (C). Sol. and Ag.S did not induce CDC (B). In HG-3 cells the Ag.R induced CDC significantly better than RTX (D), and no difference was shown in cells viability after incubation with Sol. or Ag.S (E) and Ab. or Ag.Ab (E).

Summary and conclusion

- Aggregated RTX didn't improve CDC in CLL B-cells with low levels of surface CD-20, excluding Ag.R that improves CDC in HG-3 cells. This can possibly be a result of formation of huge aggregates that caused steric interference and blockage the RTX binding sites. In addition, the low percentage of aggregates in the sample, didn't confer superior CDC efficacy
- Separation of the aggregates from monomers and new aggregation methods may be used to improve the results.