Antibody-Dependent Complement Responses towards SARS-CoV-2 Receptor-Binding Domain Immobilized on "Pseudovirus-like" Nanoparticles

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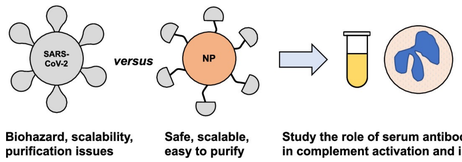
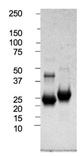
INTRODUCTION

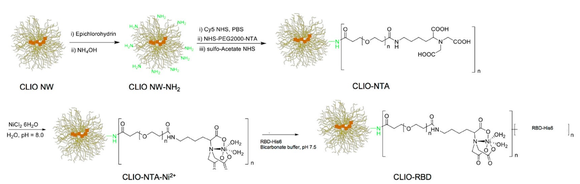
- Complement is the critical arm of the innate immunity responsible for neutralization of pathogens and a plethora of foreign particulates.
- The contribution of the complement system to SARS-CoV-2 infection and the pathology of COVID-19 is still actively debated, but some aspects of complement activation are associated with worsening of the clinical outcome.
- It is interesting to explore the role of emerging neutralizing antibodies against the receptor-binding domain (RBD) of SARS-CoV-2 in complement activation and opsonization.
- We introduce "pseudovirus-like" nanoparticles with ~70 copies of functional recombinant RBD as a simpler, safer and more scalable alternative to virion to study complement activation, C3 opsonization, and immune recognition in full human serum/blood.



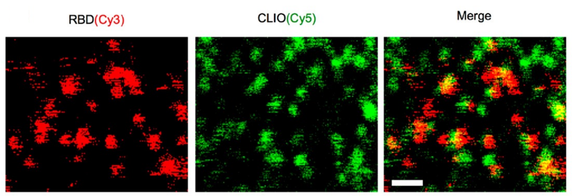
"PSEUDOVIRUS-LIKE" NANOPARTICLES



Purified His-tagged RBD (left to right: nonreduced and reduced forms)



Synthesis of CLIO-RBD by conjugating recombinant RBD of SARS-CoV-2 to crosslinked dextran iron oxide nanoworms (CLIO NWs) via Ni2+/NTA chemistry



High magnification confocal microscopy of CLIO (Cy5) - RBD (Cy3)

RESULTS

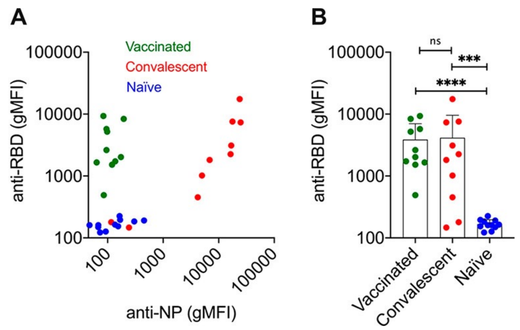


Figure 1. Anti-RBD and anti-N-protein level in donors' sera. A) Anti-RBD and anti-N-protein antibody (IgG) levels (geometric mean fluorescence intensity (gMFI)) by flow cytometry-cased immunoassay. B) Comparison of anti-RBD levels in 3 donor groups (n-10 vaccinated, 10 convalescent, and 11 naïve donors, ***p<0.001;****p<0.0001).

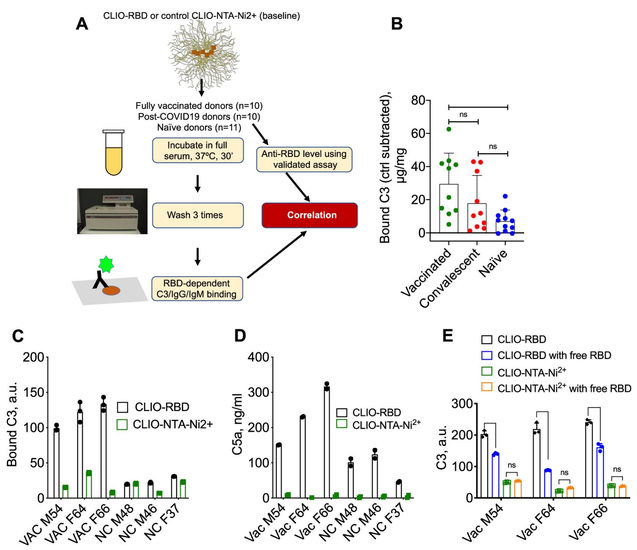
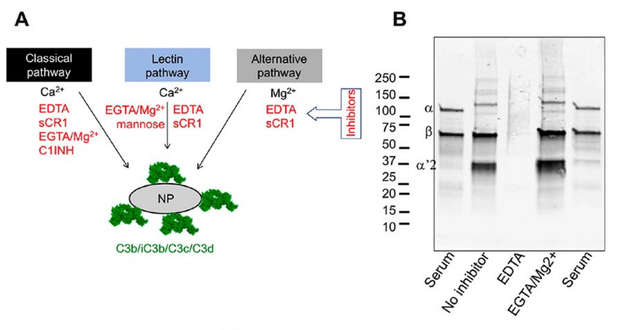


Figure 2. RBD-dependent C3 deposition on nanoparticles. A) Study design. C3, IgG, and IgM binding were quantified by dot-blot assay. B) Levels of bound C3 (µg C3/mg Fe) were calculated after subtracting C3 deposition on control CLIO-NTA-Ni2+ particles. C, D) Deposition of C3 (C) and release of fluid phase marker C5a (D) after incubation of CLIO-RBD and CLIO-NTA-Ni2+ unvaccinated (VAC) and naïve (NC) sera. E) C3 deposition on CLIO-RBD is decreased in the presence of 0.2 mg/mL soluble RBD protein (****p < 0.0001).

RESULTS



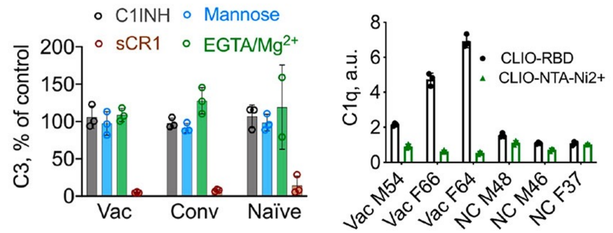


Figure 3. C3 deposition via IgG is alternative pathway-driven. A) Three complement pathways converge into C3 cleavage and nanoparticle opsonization by C3 fragments (C3b/iC3b/C3c/C3d). Inhibitors for each pathway are shown in red. B) Western blot analysis of nanoparticle-deposited C3 in vaccinated serum. C) Complement inhibition results (% of serum control) in donors with the highest RBD-dependent C3 deposition showing that CP and LP are not involved in C3 opsonization. C1INH, 100 µM; sCR1, 1 µM; mannose, 250 µM. D) Dot-blot analysis of binding of C1q showing increased binding to CLIO-RBD in vaccinated sera, but the binding was extremely low and did not lead to activation of the classical pathway.

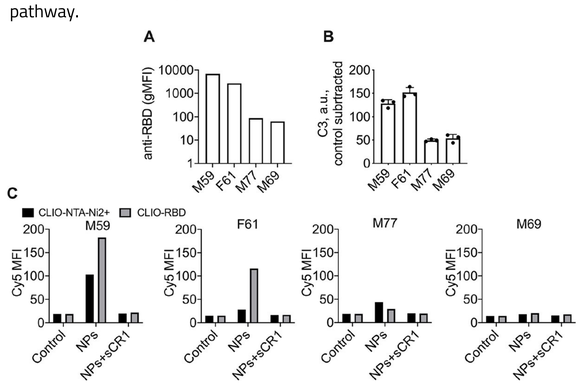


Figure 4. Variable uptake of "pseudovirus-like" nanoparticles by leukocytes in lepirudin anticoagulated blood. A) Blood donors with high and low anti-RBD titers measured with the microbead assay. B) RBD-dependent C3 deposition in plasma. C) Uptake of nanoparticles by total leukocytes and effect of complement inhibitor sCR1 on uptake in 4 blood donors.

RESULTS

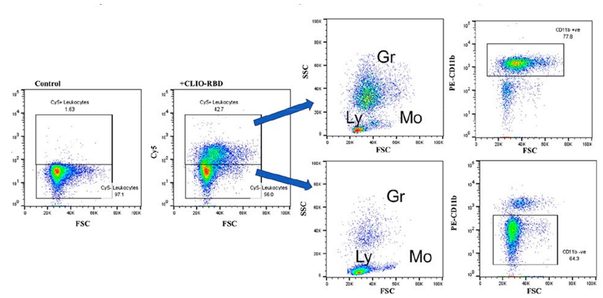


Figure 5. Leukocytes uptake in lepirudin anticoagulated blood. (D) Flow analysis of uptake by leukocytes in blood from donor F61. Ly = lymphocytes; Mo = monocytes; Gr = granulocytes.

CONCLUSIONS

- Nanoparticles fix complement in RBD-dependent manner in sera of all vaccinated, convalescent, and naïve donors, but vaccinated and convalescent donors with the highest levels of anti-RBD antibodies show significantly higher IgG binding and higher deposition of the third complement protein (C3).
- The opsonization via anti-RBD antibodies is not an efficient process: on average, each bound antibody promotes binding of less than one C3 molecule.
- RBD-dependent C3 deposition is exclusively through the alternative pathway. C3 molecules bind to protein deposits, but not IgG, on the nanoparticle surface.
- "Pseudovirus-like" nanoparticles promote complement-dependent uptake by granulocytes and monocytes in the blood of vaccinated donors with high anti-RBD titers.
- Using nanoparticles displaying SARS-CoV-2 proteins, we demonstrate subject-dependent differences in complement opsonization and immune recognition.
- These "pseudovirus-like" particles improve our understanding of how SARS-CoV-2 surface proteins are recognized by the surveillance network of the innate immunity in a relevant biological milieu.

REFERENCE

Gaikwad, H., Li, Y., Wang, G., Li, R., Dai, S., Rester, C., ... & Simberg, D. (2022). Antibody-Dependent Complement Responses toward SARS-CoV-2 Receptor-Binding Domain Immobilized on "Pseudovirus-like" Nanoparticles. ACS nano 2022.

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