Binding and neutralizing activity of a dimeric IgA version of an oligomannose-specific broadly neutralizing antibody to HIV-1

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Background

- Broadly neutralizing antibodies (bnAbs) to the HIV-1 envelope spike have been guiding prophylactic vaccine strategies. Potential target sites include a conserved patch of high-mannose glycans (‘HMP’).

- However, bnAbs mostly evaluated as IgG for protection against mucosal challenge in animal models. IgA has not been explored extensively.

- Some evidence of protective benefit of IgA against HIV at mucosal sites[1-3]. IgA2 of particular interest given its higher concentration in colonic and vaginal external secretions in people[4].

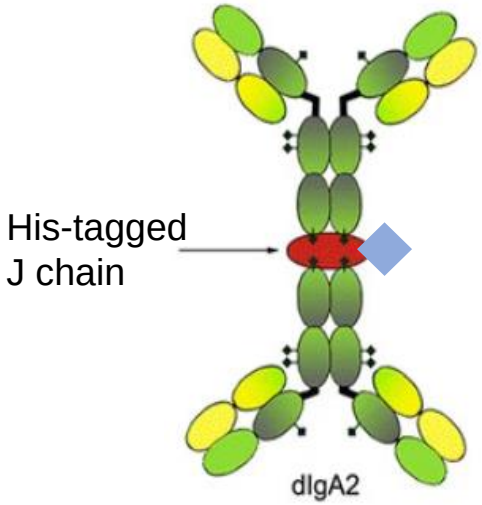


Figure 1. Schematic representation of the chemical structure of dimeric IgA2 (dIgA2). Two IgA2 monomers are dimerized in end-to-end fashion. His-tag was added to J chain by mutagenesis.

In this study, we explored conditions for successful expression of a HMP-specific bnAb (PGT128) in a dimeric IgA2 format and evaluated its HIV-neutralizing activity relative to the IgG form

Materials and Methods

Protein expression and purification

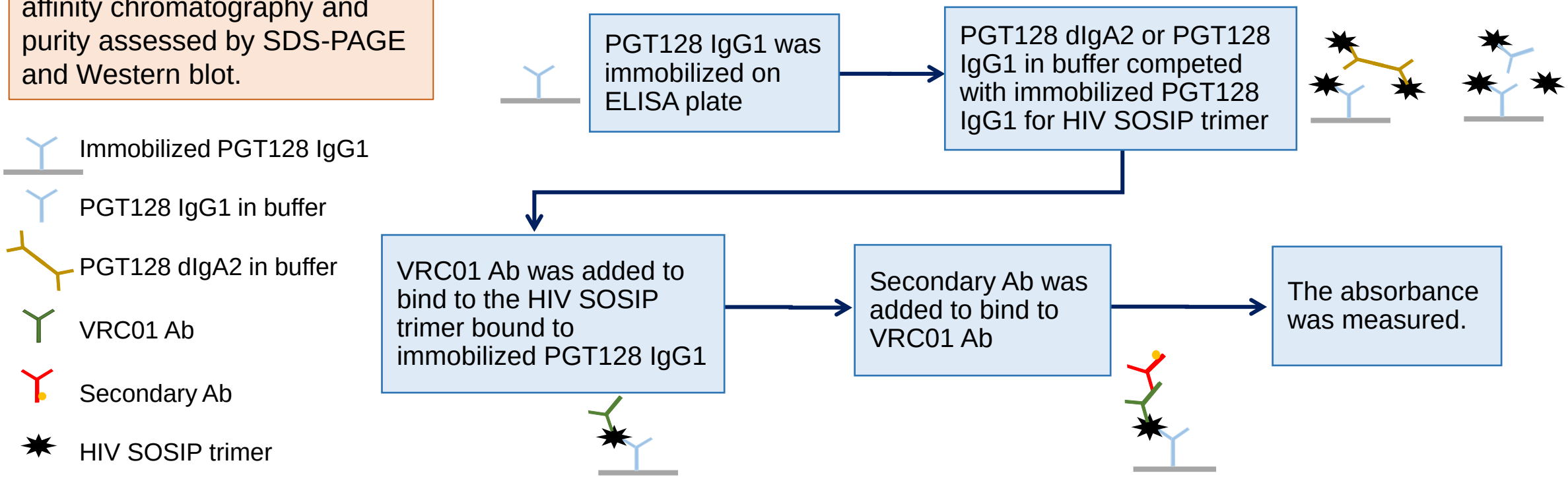
Different ratios of antibody and J chain plasmids in transfection tested to increase expression of PGT128 dIgA2 in FreeStyle 293F cells. Antibody was purified by light-chain specific affinity chromatography and purity assessed by SDS-PAGE and Western blot.

ELISA binding competition assay

Used CD4-binding site specific antibody (VRC01) as reporter to compare avidity of PGT128 dIgA2 and PGT128 IgG1 for SOSIP trimers.

Virus neutralization assay

Neutralizing activity of PGT128 dIgA2 in comparison to IgG1 assessed in pseudovirus-based assay with luciferase reporter.



Results (I)

Protein expression and purification

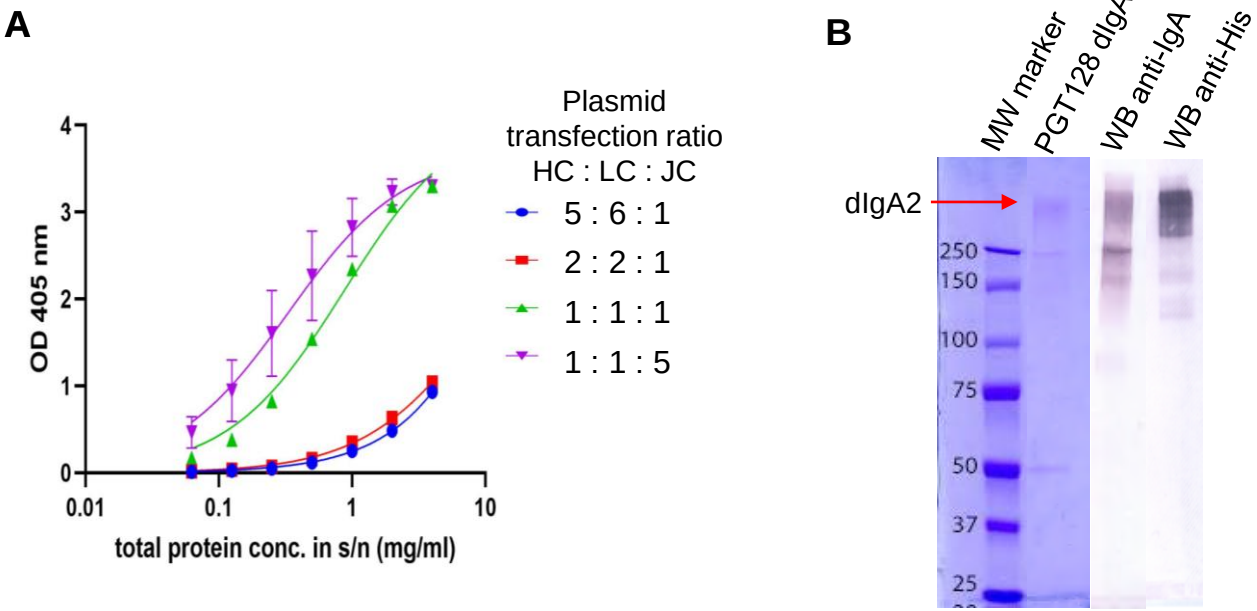


Figure 2: PGT128 dIgA2 expression is improved by increasing the ratio of transfected J chain plasmid. (A) A capture ELISA was used to detect the overall expression level of PGT128 dIgA2. An anti-IgA mAb was used for capture and bound dIgA2 detected with an anti-HIS antibody via the HIS-tagged J chain. The plasmid ratios for heavy chain (HC), light chain (LC), and HIS-tagged J chain (JC) are denoted. (B) Assessment of dIgA2 purity by SDS-PAGE (left) and confirmation of protein identity by Western blot (right) for the transfection with HC:LC:JC ratio of 1:1:5 using anti-IgA and anti-HIS Abs.

ELISA binding competition assay

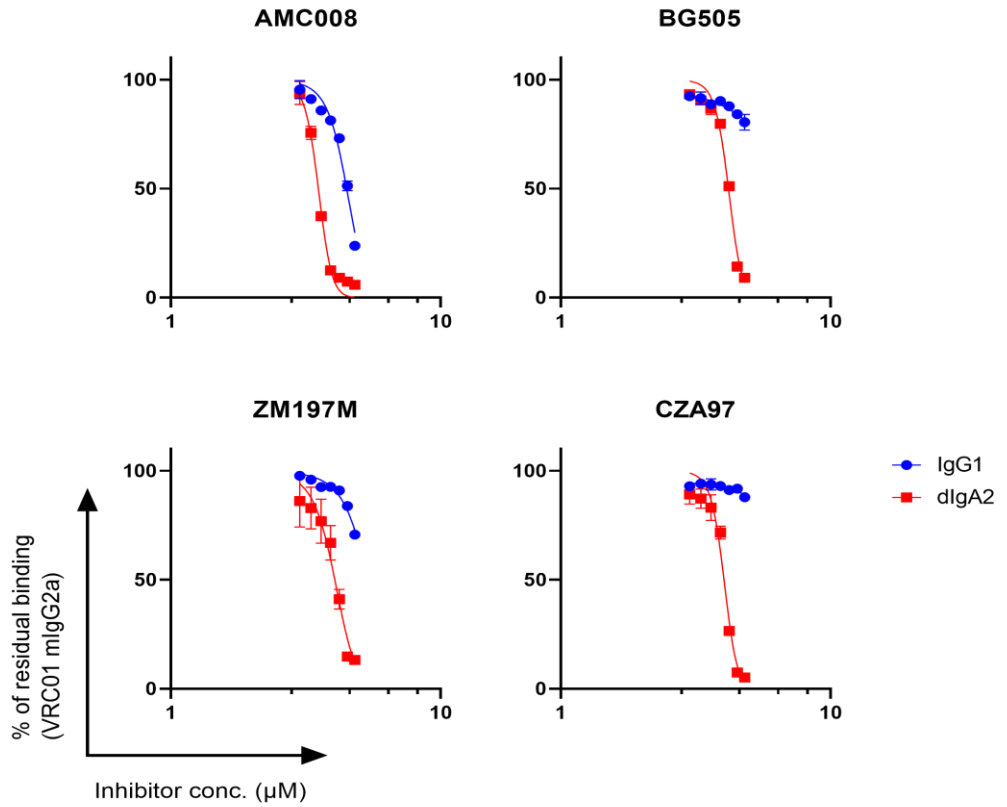
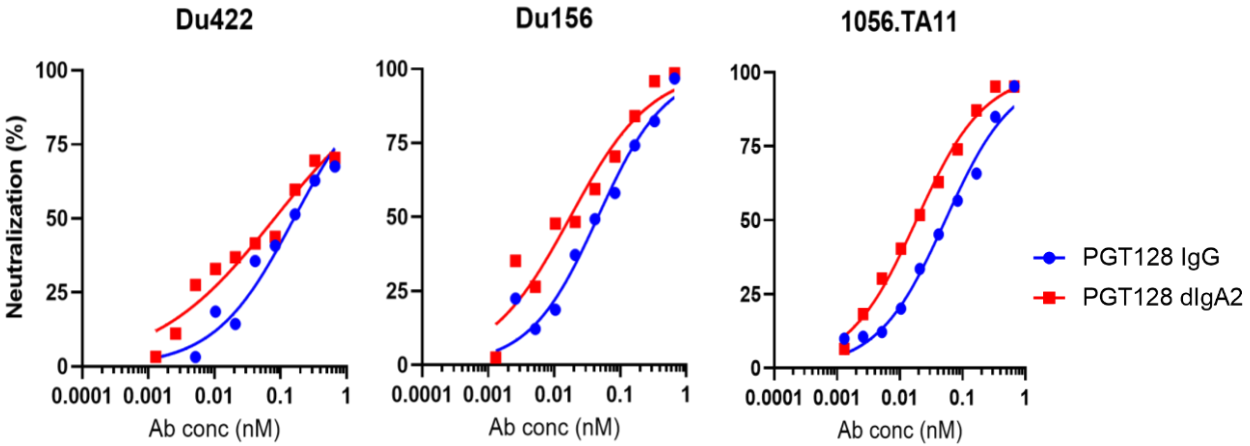


Figure 3: PGT128 dIgA2 binds recombinant HIV trimers more avidly than PGT128 IgG1. SOSIP trimers at fixed concentration (10 nM) were incubated with titrated concentrations of PGT128 IgG1 or dIgA2, then added to PGT128-coated ELISA plates and the level of bound trimer determined with VRC01, which is specific for the CD4-binding site and does not obstruct PGT128 binding.

Results (II)

Virus neutralization assay



	PGT128 IgG	PGT128 dIgA2
IC50	0.1603	0.08139

	PGT128 IgG	PGT128 dIgA2
IC50	0.04588	0.01750

	PGT128 IgG	PGT128 dIgA2
IC50	0.05314	0.01835

Figure 4: PGT128 dIgA2 neutralizes select HIV strains with 2- to 3-fold greater potency than PGT128 IgG1. Antibody neutralizing activity was assessed against two subtype C viruses (Du422, Du156) and a subtype B virus (1056.TA11). The viruses were selected for their known sensitivity to PGT128 IgG. IC50 values (nM) are denoted below each graph.

References

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Summary and Conclusions

- Increasing ratio of plasmid encoding J chain in transfection improves formation of dIgA2.
- The ELISA binding competition assays indicate increased binding avidity of PGT128 dIgA2 for native-like HIV envelope trimer compared to the IgG1.
- PGT128 dIgA2 exhibits somewhat better neutralization than IgG1, suggesting some benefit of dIgA2 valency.
- Results support further evaluation of PGT128 IgA for protection and potential implications for strategies aimed at eliciting HMP-targeted bnAbs.