Molecular Mechanisms Underlying anti-HIV-1 Fc-Effector Functions of Human IgA Allelic Variants

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420

BACKGROUND

IgA is the most abundant antibody (Ab) in humans, yet its function in viral infections, including HIV-1, remains elusive. Humans have two IgA subclasses: IgA1 (with 1 allelic variant) and IgA2 (with 3 allelic variants), differing in sequence, hinge length, and glycosylation, and expressed as monomers (mIgA), dimers (dIgA), or secretory forms (sIgA). Neutralization at mucosal surfaces is believed to be the primary anti-viral mechanism of IgA. Yet, while all IgA forms have an Fc region, only mIgA is known to bind FcαRI (CD89) and mediate Antibody-Dependent Cellular Phagocytosis (ADCP) and Antibody-Dependent Cellular Cytotoxicity (ADCC). dIgA and sIgA are believed to lack significant Fc-effector function due to steric hindrance of accessible CD89 binding sites. Here we aim to provide a comprehensive structure-anti-HIV-1 Fc-function analysis of all known variants of human IgA.

METHODS

Monomeric IgA (mIgA), dimeric IgA (dIgA), and secretory IgA (sIgA) forms of IgA1 and IgA2 were generated using the variable regions of anti-HIV-1 IgG1 Abs: **A32**, the non-neutralizing Ab (nnAb) that recognizes a highly conserved epitope within and the constant region 1 and 2 (C1-C2) of the gp120 inner domain (referred to as the Cluster A region[1]) that is capable of potent ADCC of CD4 inducible (CD4i) targets and **N49P9.6 FR3**, broadly neutralizing Ab (bnAb) specific for CD4 binding site[2]. CD89 binding was evaluated by surface plasmon resonance (SPR) and fluorescence correlation spectroscopy (FCS). CD89-mediated signaling through Syk and ERK kinase phosphorylation was analyzed using a multiplex Luminex assay. ADCP of virions and ADCC of gp120-coated cells were measured using neutrophils or peripheral blood mononuclear cells (PBMCs) as effector cells, respectively. Structures of the Fc-FcαR complexes were solved by X-ray crystallography.

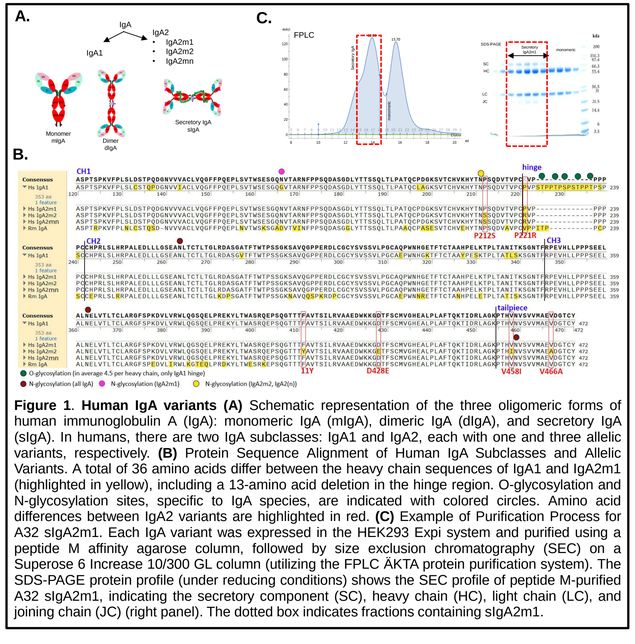


Figure 1. Human IgA variants (A) Schematic representation of the three oligomeric forms of human immunoglobulin A (IgA): monomeric IgA (mIgA), dimeric IgA (dIgA), and secretory IgA (sIgA). In humans, there are two IgA subclasses: IgA1 and IgA2, each with one and three allelic variants, respectively. **(B)** Protein Sequence Alignment of Human IgA Subclasses and Allelic Variants. A total of 36 amino acids differ between the heavy chain sequences of IgA1 and IgA2m1 (highlighted in yellow), including a 13-amino acid deletion in the hinge region. O-glycosylation and N-glycosylation sites, specific to IgA species, are indicated with colored circles. Amino acid differences between IgA2 variants are highlighted in red. **(C)** Example of Purification Process for A32 sIgA2m1. Each IgA variant was expressed in the HEK293 Expi system and purified using a peptide M affinity agarose column, followed by size exclusion chromatography (SEC) on a Superose 6 Increase 10/300 GL column (utilizing the FPLC ÄKTA protein purification system). The SDS-PAGE protein profile (under reducing conditions) shows the SEC profile of peptide M-purified A32 sIgA2m1, indicating the secretory component (SC), heavy chain (HC), light chain (LC), and joining chain (JC) (right panel). The dotted box indicates fractions containing sIgA2m1.

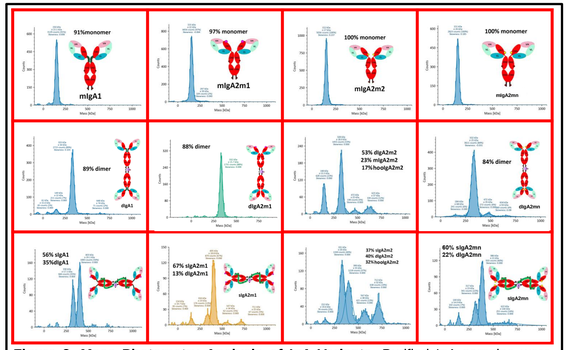


Figure 2. Mass Photometry Analysis of IgA Variants. Purified IgA samples were analyzed for mass homogeneity using a mass photometer (Refeyn Two MP). For the monomeric and dimeric variants of IgA1, IgA2m1, and IgA2mn, we detected a single peak corresponding to either the monomer or dimer, with an estimated purity of 91-100% and 84-89%, respectively. In contrast, dIgA2m2 and all sIgA variants displayed greater oligomerization heterogeneity. In the dIgA2m2 sample, we detected monomeric forms and higher-order oligomers (hooIgA2m2). For all variants of sIgA, we observed dimeric forms and higher-order oligomers, limiting sIgA content to 57-60% for sIgA1, sIgA2m1, and sIgA2mn, and only 37% for sIgA2m2.

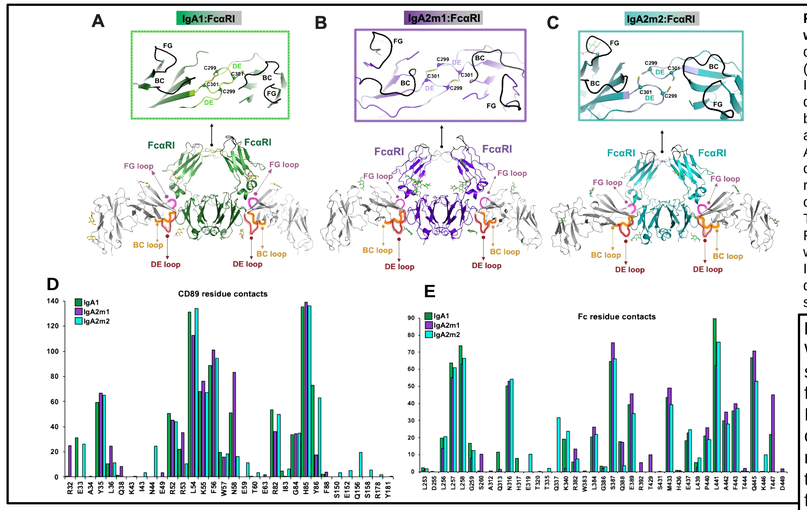


Figure 4. Crystal structures of the Fc of human IgA1, IgA2m1, IgA2m2 with FcαRI (CD89). Overall structure of the complex shown as a ribbon diagram: (**A**) IgA1, **(B)** IgA2m1 and **(C)** IgA2m2. **(D), (E)** Buried surface area (BSA) of FcαRI and IgA Fc residues buried at the complex interface. The IgA2m1 Fc-FcαRI and IgA2m2 dimers closely resemble the IgA1 Fc-FcαRI dimer. Two Fc-FcαRI molecules bind one Fc dimer near the hinge region between the $C_\alpha2$ and $C_\alpha3$ domains with a root mean square deviation for C_α atoms of 1.0 Å for the two complexes (FcαRI d1 domain and Fc monomer). Although there is a high degree of conservation of FcαRI interface residues contributing to the interface across IgA subclasses (panel **D**), the BSA of Fc residues differ more (panel **E**). Overall, IgA1, IgA2m1 and IgA2m2 forms complex with FcαRI with total BSA of 1588, 1593, 1572 Å^2 , respectively. The IgA2m1 and IgA2m2 Fc dimers have one extra glycosylation site per Fc. We also see a different disulfide bond pattern stabilizing the dimer than was seen in the initial IgA1 complex structure (PDB ID 1OW0)[4]; IgA1, IgA2m1 and IgA2m2 Fc dimers in our structures are stabilized by reciprocal disulfide bonds between Cys^{299} and Cys^{301}, while the IgA1 Fc in 1OW0 is stabilized by reciprocal bonds between Cys^{242} and Cys^{299}.

RESULTS

We engineered and expressed all known human IgA subclasses and allelic variants, along with their oligomeric forms, using the variable regions of A32 and N49P9.6 FR3 IgG1. All IgA variants bound to FcαRI, with dissociation constants in the low micromolar range (1.5-2 x 10^{-7} M). All monomeric IgA (mIgA) variants were capable of signaling through FcαRI and mediating Fc-dependent effector functions, including ADCP and ADCC, with IgA1 and IgA2mn showing slightly enhanced potency. Notably, we also observed evidence of ADCC activity with dIgA2m1 and ADCP activity for dIgA2mn. Crystal structures of the Fc-FcαRI complex for mIgA1, mIgA2m1 and mIgA2m2 revealed slight differences in the formation of the binding interfaces.

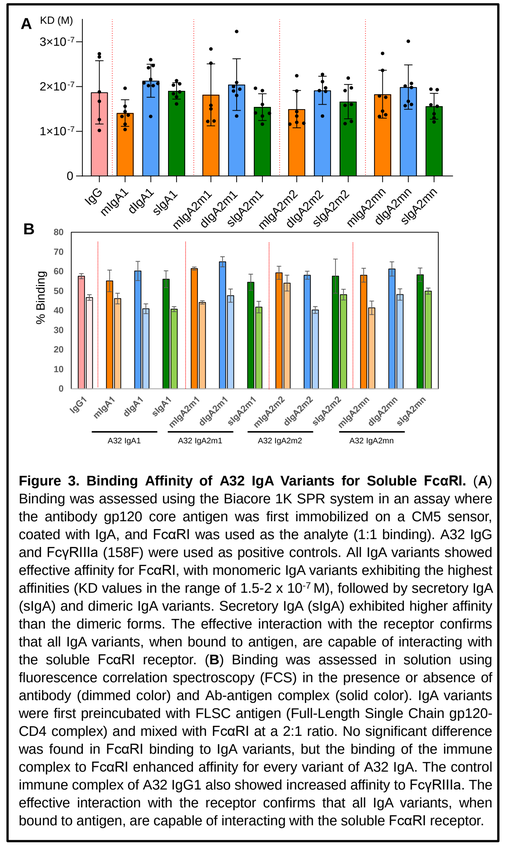


Figure 3. Binding Affinity of A32 IgA Variants for Soluble FcαRI. (**A**) Binding was assessed using the Biacore 1K SPR system in an assay where the antibody gp120 core antigen was first immobilized on a CM5 sensor, coated with IgA, and FcαRI was used as the analyte (1:1 binding). A32 IgG and FcγRIIIa (158F) were used as positive controls. All IgA variants showed effective affinity for FcαRI, with monomeric IgA variants exhibiting the highest affinities (KD values in the range of 1.5-2 x 10^{-7} M), followed by secretory IgA (sIgA) and dimeric IgA variants. Secretory IgA (sIgA) exhibited higher affinity than the dimeric forms. The effective interaction with the receptor confirms that all IgA variants, when bound to antigen, are capable of interacting with the soluble FcαRI receptor. (**B**) Binding was assessed in solution using fluorescence correlation spectroscopy (FCS) in the presence or absence of antibody (dimmed color) and Ab-antigen complex (solid color). IgA variants were first preincubated with FLSC antigen (Full-Length Single Chain gp120-CD4 complex) and mixed with FcαRI at a 2:1 ratio. No significant difference was found in FcαRI binding to IgA variants, but the binding of the immune complex to FcαRI enhanced affinity for every variant of A32 IgA. The control immune complex of A32 IgG1 also showed increased affinity to FcγRIIIa. The effective interaction with the receptor confirms that all IgA variants, when bound to antigen, are capable of interacting with the soluble FcαRI receptor.

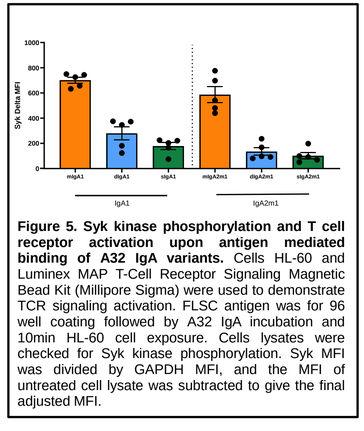


Figure 5. Syk kinase phosphorylation and T cell receptor activation upon antigen mediated binding of A32 IgA variants. Cells HL-60 and Luminex MAP T-Cell Receptor Signaling Magnetic Bead Kit (Millipore Sigma) were used to demonstrate TCR signaling activation. FLSC antigen was for 96 well coating followed by A32 IgA incubation and 10min HL-60 cell exposure. Cells lysates were checked for Syk kinase phosphorylation. Syk MFI was divided by GAPDH MFI, and the MFI of untreated cell lysate was subtracted to give the final adjusted MFI.

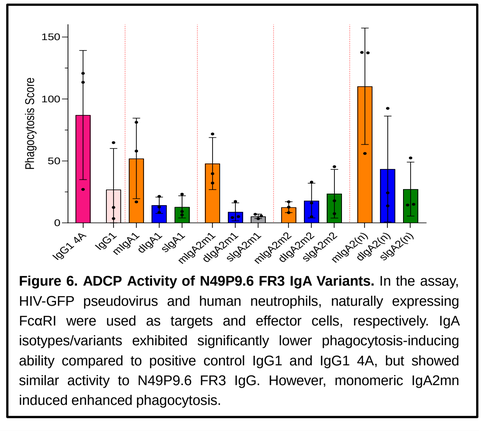


Figure 6. ADCP Activity of N49P9.6 FR3 IgA Variants. In the assay, HIV-GFP pseudovirus and human neutrophils, naturally expressing FcαRI were used as targets and effector cells, respectively. IgA isotypes/variants exhibited significantly lower phagocytosis-inducing ability compared to positive control IgG1 and IgG1 4A, but showed similar activity to N49P9.6 FR3 IgG. However, monomeric IgA2mn induced enhanced phagocytosis.

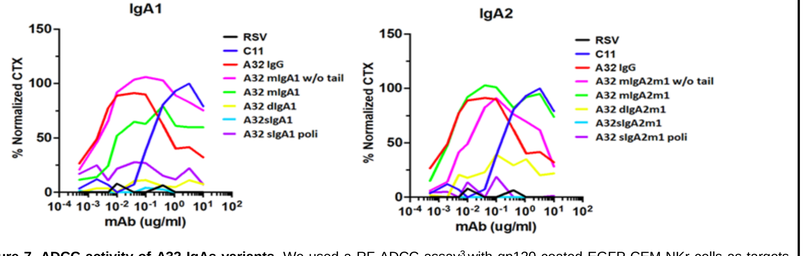


Figure 7. ADCC activity of A32 IgAs variants. We used a RF-ADCC assay[3] with gp120 coated EGFP-CEM-NKr cells as targets and PBMCs as effector cells to test the ability of each expressed IgA variant to mediate ADCC. We cloned IgA variants using variable domain of the well-known A32 IgG1. A32 IgG1 is non-neutralizing Ab but capable of potent ADCC by engaging FcγRIIIa on the effector cell surface[3]. Monomeric species of A32 IgA ware capable of ADCC activity comparable to control A32 IgG1 with A32 mIgA2m1 more potent, than A32 mIgA1. ADCC activity of A32 dIgA2m1 was moderate and no activity was found for sIgA2m1, dIgA1 and sIgA1. Altogether, these data indicate that monomeric IgA and dimeric IgA2m1 are capable of effective signaling through FcαRI to induce ADCC of antigen-coated cells.

CONCLUSIONS

1. Monomeric, dimeric, and secretory forms of all IgA subclasses/IgA2 alleles tested show effective binding to FcαRI in solution. Although the monomeric forms show the highest affinity by SPR, all variants have a KD in the micromolar range, similar to IgG1 and high-affinity FcγRs. When measured by FCS, the binding of antigen-bound Abs is elevated compared to antigen-free IgA.
2. Effective binding of all IgA variants to the FcαRI receptor in solution does not translate to biological function. Monomeric forms of all IgA variants are the most potent in triggering signal transduction pathways through the phosphorylation of Syk kinase. Monomers are also the most effective in mediating ADCP and ADCC, with mIgA2mn being the most ADCP active among all monomeric IgA subclasses/IgA2 alleles.
3. Although monomers are the most effective in signaling and downstream ADCP/ADCC processes, we detected ADCP activity for sIgA1 and sIgA2mn and moderate ADCC for sIgA2m1. This suggests that monomeric IgA is not the only form of human IgA capable of binding to and signaling through the FcαRI receptor.

References:

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