Engaging the ‘A’ Class Antibody: Variable-Heavy (VH) region influencing IgA1&2 engagement of FcαRI and superantigen proteins G, A, and L.

Wei-Li Ling^{1,2}, Chinh Tran-To Su⁵, Wai-Heng Lua¹, Joshua Yi Yeo¹, Jun-Jie Poh¹, Yuen-Ling Ng², Anil Wipat³, Samuel Ken-En Gan^{1,4,*}

1 Antibody & Product Development Lab, Experimental Drug Development Centre – Bioinformatics Institute, Agency for Science, Technology and Research (A*STAR), Singapore

2 Newcastle Research and Innovation Institute (NewRIIS), Singapore

3 School of Computing, Newcastle University, UK

4 James Cook University, Singapore

5 Bioinformatic Institute, Agency for Science, Technology and Research (A*STAR), Singapore

* Correspondence:

Samuel Ken-En Gan

Samuel_gan@eddc.a-star.edu.sg; samgan@apdskeg.com

One Sentence Summary: An insight into the mechanism of distal V-region effects on FCAR and superantigens proteins G, A, and L by both IgA1 and A2.

Abstract

Interest in IgA as an alternative therapeutic and diagnostic antibody has increased over the years, yet much remains to be investigated especially given their importance in activating immune cells in blood and in mucosal immunity. Recent whole antibody-based investigations have shown significant distal effects between the variable (V) and constant (C)- regions that can be mitigated by the different hinge regions of the human IgA subtypes A1 and A2. Diving deeper into the mechanisms underlying this, systematic VH manipulations retaining the CDRs were performed on a panel of 28 IgA1s and A2s across the Trastuzumab and Pertuzumab models, revealed distal effects on Fc α RI binding. Further insights from structural modelling showed these effects to also be mitigated by the differing glycosylation patterns in IgA1 and 2 to explain reversal of trends of IgA1s and 2s effected by slight changes in the CDRs. IgAs bound at the Fc showed similar trends but magnitudes better binding to Her2 with that bound by ppL, showing that ppL can sterically hinder Her2 antigen binding. Contrary to canonical knowledge, we found strong evidence of IgAs binding SpG that was narrowed to be at the CH2-3 region, and that the likely binding with SpA was beyond VH3 FWR and most likely at the CH1. VH1 was found to be the most suitable framework (FWRs) for CDR-grafting for both IgA1 and 2. With relevance to interactions with the microbiome at mucosal surfaces, mechanistic insight of how these IgAs can interact bacterial superantigens proteins G, A, and L are also discovered for potential future interventions.

Introduction

As the highest produced antibody (66 mg/ mL per day) making up 75% of antibodies in mucosal areas (1, 2), IgA plays a key role in protecting a vast surface area of ~400 m² (1) that includes the respiratory and gastrointestinal tracts (3). Beyond playing a key role in mucosal immunity, IgA also confers passive immunity through breastfeeding such as passing SARS-COV-2 antibodies to new-borns (4). Therapeutically, interest in IgA is increasing (5) given its efficiency to elicit antibody dependent cell-mediated cytotoxicity (ADCC) (6-8), antibody dependent cell-mediated phagocytosis (ADCP) (9), secretion of myeloperoxidase (10), reactive oxygen species (ROS) production (10), and neutrophil extracellular traps release (NETosis) (11, 12) against both infectious diseases (13-17) and ductal tumours (5, 18) in oncology.

The normal microbial flora in the mucosal areas (19) where IgA is often found, e.g. *Staphylococcus aureus*, often secrete superantigens that bind antibodies (20), preventing IgA-FcαRI interaction and the serum killing of bacteria (21) to increase susceptibility to septicemia, one of leading causes of death (22). These superantigens were also speculated to be linked to glomerulonephritis (23, 24), mimicking IgA nephropathy (25, 26), displaying their clear importance in clinical pathogenesis, and the need to prevent future therapeutic IgAs from eliciting such effects. Interventions and mitigation to such clinical pathogenesis and biologics engineering require in-depth mechanistic study while retaining superantigen-based purification in biologics manufacturing (27) for the latter. While Proteins G (SpG), A (SpA), and L (PpL) are commonly used for IgG therapeutics, the matrix choices for IgA purification are typically that of Peptide M (28), IgA-binding protein, Jacalin (27), and recently, the new Protein A/G (29).

The two subclasses: IgA1 and IgA2, differ in post-translational glycosylation patterns (30-33) and in the hinge connecting the Cα1 and Cα2 of the heavy chains. Compared to IgA2, IgA1 has a longer hinge, allowing it more flexibility for better accessibility to bind antigens

(34). IgAs exist in monomeric; dimeric; and secretory forms, with the monomeric forms predominantly found in serum as IgA1 (5) to bind Fc α RI (CD89) (35-37) on myeloid lineage cells (38-41) for immune activation (42). The dimeric forms, existing predominantly as two monomeric IgA2 conjoined at the tails of their constant (C) regions by the 16kDa J-chain protein (43, 44), are primarily found at the mucosal areas. This binding of the dimeric IgA2 to the polymeric Ig receptor (pIgR) expressed on the basolateral side of epithelial cells allows its translocation to the lumen after attachment and cleavage of the extracellular part of pIgR known as secretory component (SC). These translocated pIgR-dimeric IgA2s are called secretory IgA (sIgA) where the SC stabilizes the complex, but also sterically hinders Fc α RI binding due to the overlapping binding sites (44-46).

Distal effects between the variable (V)- to constant (C)-regions in IgGs (47) and IgE (48) were reported, in which changes in V-regions could modulate the interaction with the respective isotype FcRs with reverse effects simultaneously reported for IgAs where C-region mutations mitigated antigen binding (49, 50). Given the contrasting hinges between the two IgA isotypes, the bi-directional effects were expectedly distinct. Thus, for a more systematically holistic investigation, we grafted Pertuzumab and Trastuzumab CDRs onto seven human heavy chain V-region heavy chain families (VH1-VH7) of IgA1 and IgA2 C-regions (49) to study effects on both antigen (Her2) and receptor (Fc α RI). Since the IgA main receptor is Fc α RI with its splice variants (51, 52), the IgA- Fc α RI interaction would focus on monomeric IgAs. The in-depth understanding of this IgA-FcR mechanism by the various regions of IgA has implications beyond biologics manufacturing, purification, and engineering as safer therapeutics (53), but also to understanding mucosal immunity, clinical glomerulonephritis, and even future mucosal vaccines against mucosal infectious diseases (54, 55).

Results

Bio-Layer Interferometry (BLI) measurements of PpL and anti-IgA immobilized

Pertuzumab and Trastuzumab VH1-7 IgA1s and 2s to His-tagged Her2

Binding measurement to Her2									
Construct	Protein L Immobilization			Anti-IgA Immobilization			Differences between Biosensor		
	KD (10 ⁻⁹)	ka (10 ⁴)	kd (10 ⁻⁴)	KD (10 ⁻⁹)	ka (10 ⁴)	kd (10 ⁻⁴)	KD	ka	kd
PVH1-IgA1	7.53	11.74	8.74	0.23	32.90	0.78	~32.7x	~2.8x	~11.2x
PVH2-IgA1	13.43	15.86	21.53	2.90	32.09	9.28	~4.6x	~2.0x	~2.3x
PVH3-IgA1	9.86	10.35	10.20	0.68	33.18	2.26	~14.5x	~3.2x	~4.5x
PVH4-IgA1	8.95	8.05	7.12	0.58	23.62	1.38	~15.4x	~2.9x	~5.1x
PVH5-IgA1	50.55	9.17	46.24	7.09	27.57	19.53	~7.1x	~3.0x	~2.3x
PVH6-IgA1	36.62	3.88	13.76	6.57	11.55	7.58	~5.5x	~2.9x	~1.8x
PVH7-IgA1	17.60	8.02	13.97	2.67	24.33	6.51	~6.5x	~2.9x	~2.1x
PVH1-IgA2	7.64	14.70	11.10	0.47	29.75	1.41	~16.2x	~2.0x	~7.8x
PVH2-IgA2	10.37	14.13	14.69	3.45	27.10	9.33	~3.0x	~1.9x	~1.5x
PVH3-IgA2	8.40	11.42	9.56	0.85	28.81	2.46	~9.8x	~2.5x	~3.8x
PVH4-IgA2	7.25	8.26	5.99	0.95	20.84	1.99	~7.6x	~2.5x	~3.0x
PVH5-IgA2	32.29	11.36	36.57	7.21	25.93	18.70	~4.4x	~2.2x	~1.9x
PVH6-IgA2	28.76	4.62	13.18	6.82	11.25	7.67	~4.2x	~2.4x	~1.7x
PVH7-IgA2	16.24	8.42	13.61	2.95	21.83	6.44	~5.5x	~2.5x	~2.1x
HVH1-IgA1	1.16	10.64	1.24	0.10	39.20	0.42	~11.6x	~3.6x	~2.9x
HVH2-IgA1		PR		0.83	37.87	3.13		NIL	
HVH3-IgA1	5.6	7.26	4.06	0.35	39.68	1.38	~16.0x	~5.4x	~2.9x
HVH4-IgA1		PR		0.50	38.45	1.90		NIL	
HVH5-IgA1	4.85	7.52	3.64	0.62	38.27	2.37	~7.8x	~5.0x	~1.5x
HVH6-IgA1	4.52	7.40	3.34	0.43	39.29	1.71	~10.5x	~5.3x	~1.9x
HVH7-IgA1	5.72	6.86	3.91	0.57	34.24	1.96	~10.0x	~4.9x	~1.9x
HVH1-IgA2		PR		0.61	38.35	2.33		NIL	
HVH2-IgA2		PR		1.02	34.01	3.09		NIL	
HVH3-IgA2	5.85	7.42	4.34	0.34	40.43	1.38	~17.2x	~5.4x	~3.1x
HVH4-IgA2		PR		0.99	31.53	3.10		NIL	
HVH5-IgA2	5.23	7.50	3.86	0.57	36.56	2.10	~9.1x	~4.8x	~1.8x
HVH6-IgA2		PR		0.29	39.23	1.18		NIL	
HVH7-IgA2	5.53	7.26	4.02	0.59	36.25	2.17	~9.3x	~4.9x	~1.8x

Figure 1. BLI measurements of PpL and anti-IgA biosensor immobilized Pertuzumab (PVH) and Trastuzumab (HVH) 1-7 IgA1 and IgA2 interaction to Her2. The KD, ka and kd values of each variant are shown accordingly with the differences shown. Poor response (PR) indicates that the antibody construct did not show reliable ka and kd measurements (in triplicates). All measurements were performed in triplicates.

To systematically study the effect of the VH framework (FWR) families on antigen binding by the IgAs, the various VH family variants were paired with the same respective Pertuzumab/Trastuzumab light chain FWR V κ 1.

The Pertuzumab IgA1 and IgA2 variants showed a relatively wide range of dissociation equilibrium constant (KD) from 7.25 to 50.55 x 10⁻⁹ M (Figure 1). The best binder (by KD) among the 14 variants is PVH4-IgA2 while the weakest binder is PVH5-IgA1. Between the two antibody subtypes, there was a trend within the same VH family that IgA2 had generally better (lower) KD compared to IgA1. VH1, 3 and 4 showed KD values toward the Her2 antigen at the range of ~7 to 9 x 10⁻⁹ M while VH2 and 7 had moderate KDs with range of ~10 to 17 x 10⁻⁹ M with VH5 and 6 having the weakest (highest) KDs of ~28 and 50 x 10⁻⁹ M. The KDs differences of better binders were found to be due to the lower rate of association (ka) and dissociation (kd) while moderate and weaker binders had higher ka and kd rates.

To cross validate Pertuzumab CDR grafting effects on IgA1 and 2, the highly similar Trastuzumab with almost identical V-region FWRs was chosen for the systematic comparison.

Trastuzumab IgAs variants were observed to differ from Pertuzumab IgAs variants with a narrower range of KD values (Figure 1) between 1.16 to 5.85 x 10⁻⁹ M. The best binder among the 14 Trastuzumab variant is HVH1-IgA1 while the weakest binder of measurable reading is HVH3-IgA2. Several of the Trastuzumab IgA variants were below the detection limits: HVH2, HVH4-IgA1, HVH1, HVH2, HVH4 and HVH6-IgA2. The trend between the A1 and A2

subclasses were reversed compared to Pertuzumab with Trastuzumab IgA1s having better KDs than its counterpart IgA2s of the same VH family. Unlike the Pertuzumab IgA variants with a spectrum in Her2 binding, Trastuzumab IgAs were more polarized with variants found at more at the extremes of binding.

To rule out the possible interference, avidity and protein orientation capture effects caused by PpL capture, we also implemented biotinylated anti-IgAs bound onto streptavidin biosensors to immobilize the IgA variants at the Fc for cross-validation antigen binding measurements.

The KD range of Pertuzumab IgAs binding Her2 (Figure 1) was between 2.38 to 72.16 x 10⁻¹⁰ M which translates to ~3.0 to 32.7 times (Figure 1) that of measurements using PpL, albeit with similar trends. VH1, 3 and 4 were the better binders with lower KDs of ~2 to 9 x 10⁻¹⁰ M, VH2 and 7 with moderate KDs at ~26 to 34 x 10⁻¹⁰ M, and VH5 and 6 as the weakest binders with KD values at ~65 and 72 x 10⁻¹⁰ M. Within anti-IgA immobilization, Pertuzumab IgA1s showed a lower (better) KDs than IgA2 of the same VH family FWRs which showed a reverse trend of IgA1 and 2 from PpL-based measurements.

Using anti-IgA immobilize at the Fc for the Trastuzumab IgAs, the KD values increased by ~7.8 to 17.2 times compared to that of using PpL as was observed for the Pertuzumab IgAs (Figure 1).

BLI measurements of NTA-Ni immobilized FcαRI interacting with Pertuzumab and Trastuzumab VH1-7 of IgA1 and 2

Binding measurement to FcαRI			
Construct	NTA-Ni Immobilization		
	KD (10^{-8})	ka (10^4)	kd (10^{-4})
PVH1-IgA1	2.86	10.70	30.07
PVH2-IgA1	3.02	9.82	27.86
PVH3-IgA1	2.18	11.10	23.92
PVH4-IgA1	2.33	10.26	23.72
PVH5-IgA1	0.84	4.76	3.94
PVH6-IgA1	2.18	9.03	19.53
PVH7-IgA1	1.93	12.16	23.17
PVH1-IgA2	2.38	9.35	21.47
PVH2-IgA2	3.26	5.88	17.00
PVH3-IgA2	1.85	9.00	16.03
PVH4-IgA2	2.52	7.60	18.54
PVH5-IgA2	0.97	4.71	4.41
PVH6-IgA2	2.57	5.84	14.45
PVH7-IgA2	1.94	9.27	17.74
HVH1-IgA1	3.40	2.55	8.65
HVH2-IgA1	PR		
HVH3-IgA1	1.01	8.52	8.47
HVH4-IgA1	PR		
HVH5-IgA1	0.89	7.03	6.18
HVH6-IgA1	2.16	7.57	16.20
HVH7-IgA1	1.42	8.13	11.44
HVH1-IgA2	3.15	2.87	9.59
HVH2-IgA2	PR		
HVH3-IgA2	1.25	8.00	9.82
HVH4-IgA2	PR		
HVH5-IgA2	0.79	7.70	6.08
HVH6-IgA2	2.59	3.26	8.23
HVH7-IgA2	1.16	8.17	9.30

Figure 2. Biolayer interferometry measurements of NTA-Ni biosensor immobilized FcαRI interaction to Pertuzumab (PVH) and Trastuzumab (HVH) 1-7 IgA1 and IgA2. The KD, ka and kd values of each variant are shown accordingly. Poor response (PR) indicates that the antibody construct did not show reliable ka and kd measurements (in triplicates). All measurements were performed in triplicates.

To investigate the effects of the VH families on FcαRI engagement by the IgAs, NTA-Ni biosensor was used to first bind recombinant His-tagged FcαRI before measurements.

The KD value range of interaction with Pertuzumab IgAs were from 0.84 to 3.26×10^{-8} M (Figure 2) with the best binder as VH5-IgA1 and the weakest binder as VH2-IgA2. Both VH5 IgA1 and 2 are strong binders to FcαRI at KDs 0.84 and 0.97×10^{-8} M with kd values of 3.94 and 4.41×10^{-4} 1/s respectively while the rest of the VH families (VH1, 2, 3, 4, 6 and 7) are the weak binders with high KDs of 1.85 to 3.26×10^{-8} M. IgA1s tend to bind better to FcαRI than IgA2s of the same VH families.

As shown in Figure 2, the observed KD values range for FcαRI interacting with Trastuzumab IgAs were from 0.79 to 3.40×10^{-8} M with VH5-IgA2 as the best binder and VH1-IgA1 as the weakest binder. The interaction of FcαRI with Trastuzumab IgAs can be categorized into the strong binders (VH5 family) with KDs of 0.79 and 0.89×10^{-8} M with low kd values of 6.08 to 6.18×10^{-4} 1/s, with weak binders of VH1, 3, 6 and 7 families with KDs of 1.01 to 3.40×10^{-8} M and lastly, the non-binders: VH2 and 4. Unlike Pertuzumab variants, there is no noticeable trend of either Trastuzumab IgA1 or IgA2 within the same VH families.

BLI measurements of immobilized proteins G, L and A interacting with Pertuzumab and Trastuzumab VH1-7 of IgA1 and 2

Binding measurement to Superantigen									
Construct	Protein G Immobilization			Protein L Immobilization			Protein A Immobilization		
	KD (10 ⁻⁹)	ka (10 ⁴)	kd (10 ⁻⁴)	KD (10 ⁻¹⁰)	ka (10 ⁴)	kd (10 ⁻⁴)	KD (10 ⁻⁸)	ka (10 ⁴)	kd (10 ⁻⁴)
PVH1-IgA1	5.25	1.78	0.93	17.94	13.21	2.37	PR		
PVH2-IgA1	3.24	3.09	1.00	20.82	14.14	2.94	22.74	0.76	17.16
PVH3-IgA1	9.49	0.92	0.88	7.95	14.19	1.13	6.17	2.62	16.18
PVH4-IgA1	3.45	2.81	0.97	14.45	14.28	2.06	21.86	0.81	17.22
PVH5-IgA1	2.78	3.71	1.03	28.92	15.47	4.46	22.37	0.82	17.57
PVH6-IgA1	2.60	4.12	1.07	24.70	13.15	3.25	21.03	0.93	19.13
PVH7-IgA1	4.25	1.92	0.81	22.64	15.70	3.56	PR		
PVH1-IgA2	7.62	1.07	0.80	11.56	19.70	2.27	PR		
PVH2-IgA2	3.15	3.00	0.93	31.00	16.51	5.06	27.42	0.61	15.89
PVH3-IgA2	14.45	0.71	1.00	5.21	16.43	0.85	6.10	3.20	19.43
PVH4-IgA2	6.52	1.23	0.77	11.40	12.85	1.46	PR		
PVH5-IgA2	3.77	2.66	0.99	22.66	16.68	3.78	75.49	0.23	17.36
PVH6-IgA2	3.70	2.54	0.93	21.69	15.08	3.26	79.10	0.44	17.69
PVH7-IgA2	9.87	0.85	0.84	12.93	15.23	1.95	PR		
HVH1-IgA1	1.98	6.79	1.32	48.38	15.80	7.64	11.20	1.65	17.91
HVH2-IgA1	1.59	9.07	1.44	88.53	7.38	6.52	3.70	1.90	7.03
HVH3-IgA1	18.22	0.83	1.58	1.35	15.66	0.21	0.47	37.13	17.32
HVH4-IgA1	1.52	10.79	1.63	51.31	9.19	4.53	PR		
HVH5-IgA1	3.19	3.93	1.23	10.82	11.09	1.20	16.50	0.82	12.62
HVH6-IgA1	1.85	8.48	1.55	18.60	13.10	2.43	6.20	3.33	20.65
HVH7-IgA1	2.52	5.32	1.32	11.55	13.82	1.59	3.43	3.52	12.00
HVH1-IgA2	2.51	6.56	1.48	47.05	5.04	2.26	0.28	3.90	0.96
HVH2-IgA2	1.70	10.28	1.58	56.36	7.29	4.00	0.84	3.33	2.91
HVH3-IgA2	14.12	0.68	0.96	0.07	19.63	0.01	0.42	37.44	15.18
HVH4-IgA2	1.79	7.92	1.38	72.59	5.78	4.25	7.77	1.39	11.15
HVH5-IgA2	4.14	3.32	1.29	1.91	20.10	0.36	27.42	0.58	14.70
HVH6-IgA2	1.26	13.16	1.64	42.05	14.09	5.86	5.02	3.20	16.60
HVH7-IgA2	3.06	4.16	1.26	7.06	15.83	1.08	4.30	6.29	19.39

Figure 3. BLI measurements of immobilized proteins G, L and A interacting with Pertuzumab (PVH) and Trastuzumab (HVH) 1-7 IgA1 and IgA2. The KD, ka and kd values of each variant are shown accordingly. Poor response (PR) indicates that the antibody construct did not show reliable ka and kd measurements (in triplicates). All measurements were performed in triplicates.

To investigate the effects of the VH families on IgAs interacting with antibody superantigens with relevance to mucosal immunity and antibody purification, the interaction of all the IgAs variants with superantigens: proteins G, L and A biosensors, were measured.

The KD range of binding to SpG (Figure 3) was from 1.26 to 18.22 x 10⁻⁹ M with the best binder as HVH6-IgA2 and weakest binder as HVH3-IgA1. It is noticeable that KD calculations of Trastuzumab variants were lower (better) than Pertuzumab variants and IgA1 generally had lower readings than the corresponding IgA2 of the same VH family. All the VH3 variants, regardless of IgA1 or 2 of Pertuzumab or Trastuzumab, had consistently higher KDs than the other VH variants within the groups tested due to the poorer (lower) ka over the changes in kd measurements.

On binding to PpL, the KD values ranged from 0.07 to 88.53 x 10⁻¹⁰ M with the best binder as HVH3-IgA2 and weakest binder as HVH2-IgA1. KD values of the Pertuzumab variants were narrower/more consistent (5.21 to 31.00 x 10⁻¹⁰ M) when compared to that of the Trastuzumab variants (0.07 to 88.53 x 10⁻¹⁰ M). All VH3 variants showed the best KD among its respective groups (Pertuzumab or Trastuzumab of IgA1 or IgA2) as determined by the low kd. Apart from the VH3s, Trastuzumab VH5 and VH7 of IgA2 are also strong binders with KD values of 1.91 and 7.06 x 10⁻¹⁰ M respectively.

On binding to SpA (Figure 3), the KD values ranged from 0.28 to 79.10 x 10⁻⁸ M with the best binder HVH1-IgA2 and weakest binder PVH6-IgA2. Trastuzumab variants generally interacted with SpA (as is with SpG) better than Pertuzumab variants. The VH3 variants showed better ka measurements within the groups, especially the Trastuzumab variants (~37 x 10⁴) when compared to Pertuzumab variants (~3 x 10⁴). Both VH1 and 2 variants of Trastuzumab IgA2 were strong binders with KD value of 0.28 and 0.84 x 10⁻⁸ M respectively.

It should be noted that their lower KD values were due to lower kd measurements rather than higher ka as was observed with the VH3 variants. The VH5 variants of both Trastuzumab IgA1 and 2 had weaker binding within the respective IgA families, and both VH5 and 6 of Pertuzumab IgA2 had the highest KD (weakest binding) within the subtypes. There were a few variants with no detectable interaction with SpA such as PVH1- and 7-IgA1, PVH1-, 4-, 7-IgA2 and HVH4-IgA1 with no consistent trends by VH families.

Differential FcαRI binding between Trastuzumab and Pertuzumab IgA1 variants

Figure 4: Computational analysis of FcαRI binding by Trastuzumab/Pertuzumab IgA1 variants.

(A). Docked complexes of FcαRI (shown in colored ribbon) to Trastuzumab (VH3, VH4, and

VH5) and Pertuzumab (VH2, VH3, VH4, and VH5) IgA1 variants. The symmetrical active sites on the Fc regions are filled in black, with key active residues involved in Fc α RI contacts labelled. For simplification, only the backbone of the Trastuzumab/Pertuzumab VH3 variants are presented in white surface, whereas the different Fv and the resulting Fc α RI binding modes are colored accordingly to the VH2 (blue), VH3 (orange), VH4 (green), and VH5 (yellow) families. The attached glycans are shown in balls and sticks with atoms following the color scheme as: carbon (accordingly with those of the VH families), oxygen (red), hydrogen (white). **(B, C)**. Interaction analyses at the Fc α RI-Fc α interfaces with relative solvent accessibility surface area (SASA) of the active site residues on the IgA1-Fc regions (B) and distance matrices between the interfacial residues (C). The color scheme follows as in (A). The visualizations were generated using PyMOL v.2.3.2 (56).

Distinct Fc α RI binding kinetics measurements were observed for different IgAs VH variants (e.g., the VH2 and VH4 in comparison to VH3/VH5 in both Trastuzumab and Pertuzumab variants as shown in Figure 2) indicating the distal effects between the V- and Fc α RI binding site at the C-regions, in agreement with our previous findings (50).

In contrast to the limited motion of the truncated IgA1 Fc alone (using PDB: 1OW0), the C-region of full-length IgA1 exhibited larger dynamics motions with respect to the long and rigid proline-rich hinge (Figure S1). The increased mobility was less pronounced for IgA2 with reduced swing-like intradomain motions as observed around the IgA1 C α 2-C α 3 joint (surrounding the Fc α RI binding sites). The signal was propagated via the hinge connected by two disulfide bridges. This suggests that this varying mobility resulted in various exposures of the Fc α RI-binding sites on the C-region with respect to IgA1 or IgA2.

Among the Pertuzumab and Trastuzumab IgAs, Fc α RI binding kinetics measurements of the Trastuzumab VH2 and VH4 variants were distinct from the other VH families and were

beyond the measurement limits. To characterize the structural Fc α RI binding in these variants, we first generated Fc α RI-bound IgA1 Pertuzumab and Trastuzumab variants of VH2, VH3 (as control), VH4, and VH5 (Figure 4A), using the HADDOCK 2.4 server (57).

We performed the docking analysis first for the IgA1 variants because the long-range communications between the V- and C-regions were the most pronounced among the full IgA1, IgA2 and the truncated Fc due to the hinge variations (Figure S1), and the referenced experimental structural complex information of the Fc α RI binding modes is currently available only for IgA1, e.g. PDB: 1OW0.

Apart from the Trastuzumab IgA1 VH2 models (which resulted in docked complexes with Fc α RI-bound configurations overlapping with the modeled glycans), the reasonable Fc α RI-bound conformations to the other IgA1 models of VH2, VH3, VH4, and VH5 diverged into two different binding modes at around the Fc α RI-binding region on the IgA1-Fc with respect to Trastuzumab or Pertuzumab variants (Figure 4A). As observed in both the Trastuzumab and Pertuzumab IgA1 models, the *N*-linked glycans attached to N263 of the C α 2 domain shielded the two hydrophobic L257 and L258 residues (more pronounced in the Pertuzumab IgA1 models) that were among the reported Fc α RI-binding residues in the truncated IgA1-Fc (37), i.e. 1OW0. These occupations of the *N*-linked glycans likely had different accommodation of the Fc α RI binding to the C-region between the Trastuzumab and Pertuzumab variants. In addition, the differences of the Fc α RI binding orientations in these IgA1 models as compared to the referenced Fc α RI-Fc α complex (1OW0) were likely due to the lack of the C-terminal tail of the Fc domain in the truncated Fc of the referenced complex 1OW0.

Since hydrophobic packing was reported as a crucial factor in the Fc α RI binding to IgA1 (36), solvent accessibility surface area (SASA) was quantified to determine the contact

interfaces of these docked complexes (Figure 4B). It was shown that the hydrophobic packing core at the central IgA1 Fc were maintained with SASA<30% burying M433 and L441, and deeper buried L439 and A442 in Pertuzumab bound complexes. Distance matrices at the Fc α RI-Fc α interfaces to determine the proximity of these interfacial residues (Figure 4C) revealed two distinct interfacial contact tendencies between those Trastuzumab and Pertuzumab IgA1 variants.

In the docked complexes of the Trastuzumab IgA1 VH3, VH4, and VH5 variants, the Fc α RI interacted mostly with the Fc α polar residues S378 and/or E389. Additional interactions with E389 via electrostatics with H85 (of Fc α RI) in the VH3 and VH5 variants may have contributed to stronger Fc α RI bindings as compared to those in the VH4, in agreement to the experimental binding kinetics measurements shown in Figure 6. The essential contribution of E389 into the Fc α RI binding was also reported in a previous mutagenesis study (36).

For the Pertuzumab IgA1 variants, signature hydrophobic interactions were found at the Fc α RI-Fc α interfaces of the VH2, VH3, VH4, and VH5 variants (Figure 4C, bottom panels) such as the hydrophobic interactions of L441 (of Fc α) and Y35, F56 and/or H86 (of Fc α RI) and of F443 (Fc α) against the hydrophobic core of Y35, L54, F56 and the aliphatic K55 of the Fc α RI.

The binding contribution of the two residues L257 and L258 to the Fc α RI were not as clearly exhibited in these Trastuzumab/Pertuzumab IgA1 VH2, VH3, VH4, and VH5 variants as compared to those in the referenced truncated Fc complexed with the Fc α RI.

Superficial binding of the Fc α RI to the IgA2 C-regions

The lack of experimentally determined structures of the Fc α RI-IgA2 complexes moved us to computationally investigate the Fc α RI binding mechanism in the IgA2 variants by docking the Fc α RI to the C-regions of the Trastuzumab/Pertuzumab IgA2 VH2, VH3, VH4, and VH5 models. We performed the docking with similar references from those of the IgA1 dockings with the highly identical sequences of the IgA1 and IgA2 Fc domains (C α 2-C α 3).

Around the two additional *N*-linked glycan sites on the IgA2 C-region, i.e. N166 (C α 1) and N324 (C α 2) (32), the *N*-linked glycans were found to be more densely distributed around the C-regions, particularly at the C α 1-C α 2 joint when compared to the IgA1 models. This suggests higher structural constraints in the whole IgA2 mobility, thereby influencing the Fc α RI-binding region on the IgA2 C-region.

Apart from the Trastuzumab IgA2 VH2 variant, the other Trastuzumab/Pertuzumab IgA2 VH2, VH3, VH4, and VH5 variants formed complexes with the Fc α RI (Figure 5A) based on the selection criteria onto the resulting docked Fc α RI-IgA2 complexes (see methods).

Within the Trastuzumab IgA2 complexes, the VH3 and VH5 docked complexes shared similar Fc α RI binding modes and interfacial SASA profiles (orange and yellow in Figure 5A-B) in contrast to the VH4 variants. Distance matrices at the Fc α RI-Fc α interfaces revealed no contacts among the interfacial residues in the Trastuzumab VH4 complexes (Figure 5C).

Within the Pertuzumab IgA2 variants, the Fc α RI formed a distinct binding orientation in the VH3 docked complexes as compared to those of VH2, VH4, and VH5. In the Fc α RI-Fc α interfaces of these Pertuzumab IgA2 variant complexes, most of the contacts involved the polar residues S374 (corresponding to S387 in the IgA1) with the H85 of the Fc α RI except for Pertuzumab IgA2 VH3 complex, which had the S374 residue detected more distant (~4.3Å) from Fc α RI F56 (Figure 5C). The interfacial SASA profiles of these Pertuzumab IgA2 variants (Figure 5B) showed that the hydrophobic core of the IgA2 C-region (involving M420, L428,

A429) remained relatively intact (e.g. SASA<40%), similar to those in the Pertuzumab IgA1 variants (corresponding to M433, L441, A442, shown in Figure 4B). None of these hydrophobic residues were detected in the Fc α RI-Fc α interfaces of the Pertuzumab IgA2 docked complexes, suggesting superficial contacts of the Fc α RI at the binding surface of the Pertuzumab IgA2 variants, particularly for the VH3 variant (e.g. Figure 5A-right panel, top view) when compared to the Pertuzumab IgA1 variant complexes.

Figure 5: Computational analysis of FcαRI binding by Trastuzumab/Pertuzumab IgA2 variants.

(A). Docked complexes of FcαRI (shown in colored ribbon) to Trastuzumab (VH3, VH4, and

VH5) and Pertuzumab (VH2, VH3, VH4, and VH5) IgA2 variants. The symmetrical active sites on the Fc regions are filled in black, with key active residues involved in Fc α RI contacts labelled. For simplification, only the backbone of the Trastuzumab/Pertuzumab VH3 variants are presented in white surface, whereas the different Fv and the resulting Fc α RI binding modes are colored accordingly to the VH2 (blue), VH3 (orange), VH4 (green), and VH5 (yellow) families. The attached glycans are shown in balls and sticks with atoms following the color scheme as: carbon (accordingly with those of the VH families), oxygen (red), hydrogen (white). **(B, C)**. Interaction analyses at the Fc α RI-Fc α interfaces with relative solvent accessibility surface area (SASA) of the active site residues on the IgA2-Fc regions (B) and distance matrices between the interfacial residues (C). The color scheme follows as in (A).

Discussion

We set out to holistically study the effect of VH families on antigen (Her2) and receptor Fc α RI binding on both IgA1 and IgA2 which while highly similar, have notable differences in glycosylation and hinge regions. We found that the VH1 and VH3 FWRs together with the avoidance of VH2 and VH4 during CDR grafting to be optimal for both IgA subtypes even though VH4 was suitable for Pertuzumab but not Trastuzumab. While VH3 is often the canonical FWR of choice in antibody humanization due to its better production (47, 58), VH1 may be a better choice for IgAs given that VH3 has propensities to bind SpA (48) and nickel (59), both of which are likely antigens at mucosal areas.

The BLI testing of Pertuzumab and Trastuzumab IgA1s and 2s immobilized via PpL biosensors showed agreement with our previous work (49, 50), but the KD values of the same IgAs and 2s immobilized via biotinylated anti-IgA sensors showed increased values across all variants including those initially below reliable detection limits using PpL (Figures 1). These

results suggest that the binding of PpL on the light chain of the antibody sterically blocked Her2 binding at the CDR region. Nonetheless, this could also be an effect of avidity of the divalent IgAs given that the overall trend between the two immobilization modes to be in agreement.

From the KD values between PpL and anti-IgA immobilized methods, superantigen PpL, produced by *Finegoldia magna* (previously known as *Peptostreptococcus magnus*), may potentially destabilize IgA antigen binding given the ~3.0 to 32.7 times difference across all VH families, subtypes and CDRs (Figure 1). Given that *Finegoldia magna* is a common commensal of genitourinary tract and gastrointestinal tract (60) where IgA is the primary antibody isotype and that the microbe is a common contaminant in blood (60), its PpL binding to the majority V κ population of human antibodies highlights its danger as a pathogen. With the potential for significant superantigen-like activation of B-cells (20) and significant dampening of antigen binding, or to cross-link V κ IgEs on sensitized basophil and mast cells at the mucosal areas to cause inflammation (61-63), it can potentially cause toxic shock syndrome (64). While IgA therapeutics are still in development, antibody engineering (53) precautions against such potential microbiome interaction that can impact multiple isotypes would be important considerations to mitigate unwanted side effects, especially during clinical trials.

Apart from superantigen PpL, the binding to proteins G and A revealed surprising IgA interactions mitigated by the V-regions that are contrary to canonical textbook findings and the company product information sheets (Table S1) of these two superantigens. Our panel showed clear contrary evidence of IgAs binding strongly to both proteins G and A that is mitigated by VH-regions. Non-VH3 Trastuzumab variants bound strongly to SpA, a finding in partial agreement to a previous ELISA study (65). Since both superantigen proteins G and A are produced by the group C & G of *Streptococcus spp.* (66, 67) and *Staphylococcus aureus* (68),

respectively, and that both are part of the normal human flora, particularly at mucosal areas and skin surfaces (69), this finding expands the potential interaction with known isotypes such as IgE (48) onto potential mast cells (63). Apart from revealing other mechanisms to microbiome-antibody interactions at the mucosal area, there is now further considerations to exploit or mitigate the IgA-superantigen properties beyond therapeutics to that of diagnostics, which is increasingly explored (70).

Although the binding of SpA and the lack of in SpG to IgAs are canonical knowledge, where SpA binds only to specific populations of IgA (65) later found to be those of the VH3 family (71), our panel provided deeper insights to this. Given that our panel showed that non-VH3 IgAs could still bind SpA and that VH3 of different CDRs and CH1 subclass affected the engagement, there is clear synergistic contribution from the VH-FWRs, CDRs and CH1 for SpA-IgA binding. The contribution of CDRs to VH3 was also in agreement with our previous finding for Trastuzumab IgE (59). Although SpG is commonly known to not bind IgA, our results suggest that such interaction occur at the IgA Fc, particularly the CH2/CH3 regions given the relative constant KD that is unaffected by VH families, CDRs and IgA subclasses.

In its role to activate effector immune cells, IgA therapeutics must engage FcαRI effectively. V-regions are commonly ignored in antibody design and development in reductionist screening methods (72), yet they affect FcR binding as was with previous IgE findings to the FcεRIα (48, 59). This thus requires a whole-antibody approach during the early stages of antibody therapeutics development to avoid unwanted surprises. Within the VH families, VH5 IgAs also showed the lowest KD (best interactions) to FcαRI for both Pertuzumab and Trastuzumab in agreement to its IgE counterpart with FcεRIα. Given that VH5 is incidentally the biased VH in allergic patients (73), its propensity to engage FcεRIα stronger (48) and bind nickel (59), has potential disease pathogenic significance given its ability for longer interaction (lowest kd) with FcαRI. With both IgEs and IgAs as mucosal antibodies, and

the possible class switching to IgA2 from IgE (74, 75), there is much to investigate on VH5 FWRs in clinical allergy pathogenesis. Interactions with microbial superantigens may be of relevance to such investigations.

With regards to IgA immobilization, the increased KDs when using anti-IgA Fc immobilization compared to PpL at the Vκ1 of ~3 to 32.7 times difference clearly uniform interference by ppL at the V-regions across the variants. Detailed investigation showed the differences to be due to the kd in Pertuzumab IgAs and ka in Trastuzumab IgAs (Figure 1). The PpL binding at Vκ1-FWR1 (76) likely sterically blocked Her2 engagement.

Contrary Her2 binding trends between the Trastuzumab and Pertuzumab IgAs are likely attributed to the slightly different combination of CDRs affecting the flexibility of the IgAs conferred by the hinge (50), as well as the varying distribution of glycans (as shown in our computational models), and the different locations of their target epitopes on Her2 affecting general accessibility of the interacting V-region residues (77). Her2 contains two different epitopes with respect to Trastuzumab and Pertuzumab (Figure S2), where the Pertuzumab-binding epitope is buried deeper as compared to that of Trastuzumab. Hence, the H3 loop of the Pertuzumab CDRs might adopt a more flexible and accessible conformation for binding to the less exposed Her2 epitope as compared to those of the Trastuzumab CDRs, a possibility illustrated by the differences observed for Trastuzumab and Pertuzumab IgMs and IgG1s (78).

Varying structural arrangements of the Trastuzumab IgA1 and IgA2 from their different hinges connecting Cα1 and Cα2 domains and glycan attachments (e.g. only IgA1 has O-linked glycans at the hinge region whereas IgA2 has two additional N-linked glycans sites at N166 and N324) can cause different FcαRI binding orientations between the two Trastuzumab IgA1 and IgA2 isotypes. Occupations of the modeled glycans near the FcαRI-binding regions was used to screen the docked FcαRI-bound Trastuzumab IgAs VH2 complexes (Figure 4C and

5C) and the Fc α RI-Fc α interfaces of the Trastuzumab IgA2 VH4 complexes (Figure 5C). This demonstrated the importance of glycans in Fc α RI binding at the C-region, supporting a previous study by Steffen *et al.* (79). However, extended sampling of the glycan dynamics combining the hinge-inducing structural constraints on the full length IgAs models upon or prior to the Fc α RI binding are essential. We observed the structural shifting of a few hydrophobic residues from the hydrophobic core (e.g. A442 or F443) occurring in both the Trastuzumab IgA1/IgA2 and Pertuzumab IgA2 variants. These structural arrangements could have resulted from the induced intradomain Ca2-Ca3 motion constraints (but not limited to the modeling artifact) that might dampen Fc α RI interactions.

Apart from the indirect impact of the hinge onto the intradomain between Ca2 and Ca3 as well as the motions of the Fab region upon Fc α RI (36), we observed possible steric hindrance caused by the flexible motions of the C-terminal tail piece of the IgA1-Fc region. With these C-terminal segments forming two symmetrical unstructured tails in our IgA1 models (whereas together with the J-chains constituting stable β -sheets in dimeric IgA1, i.e. PDB: 7JG1), Fc α RI targeting the hydrophobic core of the IgA1 C-region was hindered sterically. This obstruction is absent in the truncated Fc and Fc α RI complex (1OW0 lacking the C-terminal tail piece) or alleviated in the docking attempts of the modified Trastuzumab IgA1 VH3 variant (with the C-terminal tail removed), i.e. similar Fc α RI binding orientation as observed in the 1OW0 being reproduced in one of the resulting docked clusters (5/121 conformations, data not shown), affirming Fc α RI binding sensitivity to the structural arrangements of IgAs.

Holistically, the similarities and differences in the trends of IgAs across the VH families show the importance of the systematic investigation of individual antibodies. It is shown here that generalizations of VH3 for superantigen bindings do not necessary apply across the board and that while IgA2s may play a smaller role in blood Fc α RI immune cell activation than IgA1s, there are contributions by the V-regions that can cause IgA2 to interact better. VH1 as

the VH-FWR of choice for IgA1 and 2 therapeutics to retain antigen and Fc α RI engagement, even though VH3 remains a good candidate for humanization. On the other hand, our findings here agree with our previous investigations on other antibody isotypes (IgE) that the VH regions can affect FcR engagement and potentially play a role in disease pathogenesis with influence from superantigens. With the demonstration of the influence of PpL on IgA engagement of antigens and strong interactions between SpG and IgAs, the molecular mechanisms that underlie the interaction of normal flora at mucosal areas to mucosal antibodies IgA are better understood for future interventions.

Materials and Methods

Recombinant protein production

All Trastuzumab and Pertuzumab VH and V κ sequences used were described previously (47). The genes were transformed into competent *E. coli* (DH5 α) strains (80) followed by plasmid extraction (Biobasic Pte Ltd) and sub-cloning into pTT5 vector (Youbio, China) using restriction enzyme sites, as previously performed (47-49, 58, 76).

Transfection, production, and purification and were performed as described previously (47, 58).

Binding Affinity Quantification

For Her2 binding, IgA variants were immobilized using PpL biosensor (Sartorius, Cat: 18-5185) or biotinylated anti-IgA antibody (Thermo Scientific, Cat: 7102882500) bound on streptavidin biosensor (Sartorius, Cat: 18-5119) and subjected to free floating Her2 to obtain the rate of association (k_a), dissociation (k_d), and equilibrium dissociation constant (KD). The program and steps used were as previously described (47-50, 58, 59, 76).

His-tagged Fc α RI (Sinobiological, Cat: 10414-H08H) were immobilized via Ni-NTA biosensor (Sartorius, Cat: 18-5101) and subject to free floating IgA variants to obtained k_a , k_d , and KD. The program and steps used were as previously described (47-50, 58, 59, 76).

Modeling of full length IgA1 and IgA2 Pertuzumab and Trastuzumab VH2, VH3, VH4 and VH5 variants

The Fv (pairing VHx-V κ 1) models of the Pertuzumab and Trastuzumab VH2, VH3, VH4, and VH5 variants were first constructed using the online ROSIE Antibody protocol (81). For those models classified as “failed” by the ROSIE (e.g. Trastuzumab VH2 and VH4 variants) due to the server overload, the standalone Rosetta package (82) was used for re-grafting. It was shown that results from both ROSIE and Rosetta standalone versions were ~87% correlated (p-value << 0.0001), hence affirming the consistency of the computational CDRs grafting and modeling between the two versions. The lowest scored grafted Fv model of each Pertuzumab/Trastuzumab VHx variant was selected for computational joining onto the heavy chain C-region (C α 1-C α 3) backbone of the IgA1 and IgA2, previously constructed (50). C κ was used for the light chain constant region.

N-linked glycans (referenced from PDB: 1OW0) were attached to several asparagine residues of the full-length IgAs models, i.e. at N263 and N459 of the IgA1. Since IgA2 contains two additional conserved N-glycan sites (32), the N-linked glycans were attached at N166 and N324 together with N251 and N446 of the IgA2 models. In addition, O-linked glycans (α 2,3-linked to Gal and α 2,6-linked to GalNAc) were attached to serine/threonine of the proline-rich hinge of the IgA1 at T228, S230, S232, T233, T236, and S240 (32, 83). The attachment of glycans was performed using CHARMM GUI *Glycans Reader & Modeler* (84). All the full-

length glycan-attached IgAs models were then energy-minimized using GROMACS v2019 (85).

Molecular docking of IgAs Pertuzumab and Trastuzumab VH2, VH3, VH4, and VH5 variants and FcαRI

The FcαRI domain was first extracted from the crystal structure of human FcαRI-bound IgA1-Fc (PDB: 1OW0) and independently docked to the C-region (chain A) of each IgA1/IgA2 Pertuzumab and Trastuzumab VH2, VH3, VH4, and VH5 models using HADDOCK 2.4 server (57) with default settings. The binding regions (active residues) on IgA1 (IgA2) are L256 (L243), L257 (L244), L258 (L245), R382 (R369), S387 (S374), E389 (E376), M433 (M420), E437 (E424), L439 (L426), L441 (L428), A442 (A429), F443 (F430), Q445 (Q432) and on FcαRI are R52, R53, L54, K55, F56, Y35, G84, H85, R82. These residues were derived from Herr *et al* (36, 37).

Since the HADDOCK server accepts only standard residues for protein-protein docking, all the glycans were first removed prior to the docking. However, the original positions of all glycans were later used to assess the resulting docked complex conformations.

Only docked FcαRI-IgAs complexes that satisfied the following criteria were selected: (i) there is no overlap between the resulting FcαRI conformation and the modeled glycans, (ii) the resulting FcαRI bound conformation reflects the central hydrophobic cores of L258, L441, M433, and F443 (of the IgAs-Fc) recruiting the packing residues Y35, F56, and H85 of the FcαRI, as previously reported (37). When more than one docked cluster conformations were satisfied with the criteria above, the cluster with better HADDOCK score (higher rank) was selected and its top 3 conformations were used as replicates for further analyses. The contacts

at the Fc α RI-Fc α interfaces of the resulting docked complexes were then determined using CIPS (86).

Acknowledgment

This work was partially supported by the Joint Council Office, Agency for Science, Technology, and Research, Singapore under Grant number JCO1334i00050 and the National Research Foundation (NRF) Singapore grant to Experimental Drug Development Centre (EDDC). The simulations were performed on the Bioinformatics Institute, A*STAR server and the petascale computer cluster ASPIRE-1 at the National Supercomputing Center (NSCC) of Singapore.

Author Contribution

Conceptualization, W.L.L. and S.K-E.G.

Methodology, W.L.L. and S.K-E.G.

Investigation, W.L.L. and C.T.T.S.

Validation, W.H.L., J.J.P. and J.Y.Y.

Writing – Original Draft, W.L.L., C.T.T.S. and S.K-E.G

Writing – Review & Editing, W.L.L., C.T.T.S., Y.L.N. and S.K-E.G

Funding Acquisition, S.K-E.G.

Supervision, S.K-E.G., Y.L.N. and A.W.

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. J. M. Woof, M. A. Kerr, The function of immunoglobulin A in immunity. *The Journal of Pathology* **208**, 270-282 (2006).
2. A. J. Macpherson, M. B. Geuking, K. D. McCoy, Homeland Security: IgA immunity at the frontiers of the body. *Trends in Immunology* **33**, 160-167 (2012).
3. B. D. Wines, P. M. Hogarth, IgA receptors in health and disease. *Tissue Antigens* **68**, 103-114 (2006).
4. S. H. Perl *et al.*, SARS-CoV-2-Specific Antibodies in Breast Milk After COVID-19 Vaccination of Breastfeeding Women. *JAMA* **325**, 2013-2014 (2021).
5. A. Breedveld, M. van Egmond, IgA and FcαRI: Pathological Roles and Therapeutic Opportunities. *Frontiers in Immunology* **10**, (2019).
6. R. Braster, T. O'Toole, M. van Egmond, Myeloid cells as effector cells for monoclonal antibody therapy of cancer. *Methods* **65**, 28-37 (2014).
7. J. E. Bakema *et al.*, Targeting FcαRI on Polymorphonuclear Cells Induces Tumor Cell Killing through Autophagy. *The Journal of Immunology* **187**, 726-732 (2011).
8. M. van Egmond, J. E. Bakema, Neutrophils as effector cells for antibody-based immunotherapy of cancer. *Seminars in Cancer Biology* **23**, 190-199 (2013).
9. J. Golay *et al.*, Glycoengineered CD20 antibody obinutuzumab activates neutrophils and mediates phagocytosis through CD16B more efficiently than rituximab. *Blood* **122**, 3482-3491 (2013).
10. R. J. Pleass, M. L. Lang, M. A. Kerr, J. M. Woof, IgA is a more potent inducer of NADPH oxidase activation and degranulation in blood eosinophils than IgE. *Molecular Immunology* **44**, 1401-1408 (2007).
11. E. Aleyd *et al.*, IgA Enhances NETosis and Release of Neutrophil Extracellular Traps by Polymorphonuclear Cells via Fcα Receptor I. *The Journal of Immunology* **192**, 2374-2383 (2014).
12. S. Arelaki *et al.*, Gradient Infiltration of Neutrophil Extracellular Traps in Colon Cancer and Evidence for Their Involvement in Tumour Growth. *PLOS ONE* **11**, e0154484 (2016).
13. M. B. Mazanec, C. S. Kaetzel, M. E. Lamm, D. Fletcher, J. G. Nedrud, Intracellular neutralization of virus by immunoglobulin A antibodies. *Proceedings of the National Academy of Sciences* **89**, 6901-6905 (1992).
14. M. B. Mazanec, C. L. Coudret, D. R. Fletcher, Intracellular neutralization of influenza virus by immunoglobulin A anti-hemagglutinin monoclonal antibodies. *Journal of Virology* **69**, 1339-1343 (1995).
15. M. B. Mazanec *et al.*, in *Advances in Mucosal Immunology: Part A*, J. Mestecky *et al.*, Eds. (Springer US, Boston, MA, 1995), pp. 651-654.
16. C. Devito *et al.*, Mucosal and Plasma IgA from HIV-1-Exposed Uninfected Individuals Inhibit HIV-1 Transcytosis Across Human Epithelial Cells. *The Journal of Immunology* **165**, 5170-5176 (2000).
17. B. Corthésy *et al.*, Rotavirus Anti-VP6 Secretory Immunoglobulin A Contributes to Protection via Intracellular Neutralization but Not via Immune Exclusion. *Journal of Virology* **80**, 10692-10699 (2006).

18. J. E. Bakema, M. van Egmond, Immunoglobulin A. *mAbs* **3**, 352-361 (2011).
19. A. J. Brosnahan, P. M. Schlievert, Gram-positive bacterial superantigen outside-in signaling causes toxic shock syndrome. *The FEBS Journal* **278**, 4649-4667 (2011).
20. A. M. Deacy, S. K.-E. Gan, J. P. Derrick, Superantigen Recognition and Interactions: Functions, Mechanisms and Applications. *Frontiers in Immunology* **12**, (2021).
21. R. Langley *et al.*, The Staphylococcal Superantigen-Like Protein 7 Binds IgA and Complement C5 and Inhibits IgA-Fc α RI Binding and Serum Killing of Bacteria. *The Journal of Immunology* **174**, 2926-2933 (2005).
22. C. Rhee *et al.*, Prevalence, Underlying Causes, and Preventability of Sepsis-Associated Mortality in US Acute Care Hospitals. *JAMA Network Open* **2**, e187571-e187571 (2019).
23. A. Koyama *et al.*, Glomerulonephritis associated with MRSA infection: A possible role of bacterial superantigen. *Kidney International* **47**, 207-216 (1995).
24. E. Wehbe, C. Salem, J. F. Simon, S. D. Navaneethan, M. Pohl, IgA-dominant Staphylococcus infection-associated glomerulonephritis: case reports and review of the literature. *NDT Plus* **4**, 181-185 (2011).
25. A. A. Satoskar *et al.*, *Staphylococcus* Infection-Associated Glomerulonephritis Mimicking IgA Nephropathy. *Clinical Journal of the American Society of Nephrology* **1**, 1179-1186 (2006).
26. S. H. Nasr, V. D. D'Agati, IgA-Dominant Postinfectious Glomerulonephritis: A New Twist on an Old Disease. *Nephron Clinical Practice* **119**, c18-c26 (2011).
27. T. D. Pack, Purification of Human IgA. *Current Protocols in Immunology* **38**, 2.10B.11-12.10B.17 (2000).
28. C. Sandin *et al.*, Isolation and Detection of Human IgA Using a Streptococcal IgA-Binding Peptide. *The Journal of Immunology* **169**, 1357-1364 (2002).
29. M. Eliasson *et al.*, Chimeric IgG-binding receptors engineered from staphylococcal protein A and streptococcal protein G. *Journal of Biological Chemistry* **263**, 4323-4327 (1988).
30. M. Tomana, W. Niedermeier, J. Mestecky, F. Skvaril, The differences in carbohydrate composition between the subclasses of IgA immunoglobulins. *Immunochemistry* **13**, 325-328 (1976).
31. T. S. Mattu *et al.*, The Glycosylation and Structure of Human Serum IgA1, Fab, and Fc Regions and the Role of N-Glycosylation on Fc γ 1 Receptor Interactions *. *Journal of Biological Chemistry* **273**, 2260-2272 (1998).
32. L. Royle *et al.*, Secretory IgA N- and O-Glycans Provide a Link between the Innate and Adaptive Immune Systems *. *Journal of Biological Chemistry* **278**, 20140-20153 (2003).
33. E. Tarelli, A. C. Smith, B. M. Hendry, S. J. Challacombe, S. Pouria, Human serum IgA1 is substituted with up to six O-glycans as shown by matrix assisted laser desorption ionisation time-of-flight mass spectrometry. *Carbohydrate Research* **339**, 2329-2335 (2004).
34. M. KILIAN, J. REINHOLDT, H. LOMHOLT, K. POULSEN, E. V. G. FRANDSEN, Biological significance of IgA1 proteases in bacterial colonization and pathogenesis: critical evaluation of experimental evidence*. *APMIS* **104**, 321-338 (1996).
35. B. D. Wines, C. T. Sardjono, H. M. Trist, C.-S. Lay, P. M. Hogarth, The Interaction of Fc α RI with IgA and Its Implications for Ligand Binding by Immunoreceptors of the Leukocyte Receptor Cluster. *The Journal of Immunology* **166**, 1781-1789 (2001).
36. M. T. Posgai *et al.*, Fc α RI binding at the IgA1 C_H2-C_H3 interface induces long-range conformational changes that are transmitted to the hinge region. *Proceedings of the National Academy of Sciences* **115**, E8882-E8891 (2018).
37. A. B. Herr, E. R. Ballister, P. J. Bjorkman, Insights into IgA-mediated immune responses from the crystal structures of human Fc α RI and its complex with IgA1-Fc. *Nature* **423**, 614-620 (2003).

38. E. Aleyd, M. H. Heineke, M. van Egmond, The era of the immunoglobulin A Fc receptor FcαRI; its function and potential as target in disease. *Immunological Reviews* **268**, 123-138 (2015).
39. R. C. Monteiro, H. Kubagawa, M. D. Cooper, Cellular distribution, regulation, and biochemical nature of an Fc alpha receptor in humans. *Journal of Experimental Medicine* **171**, 597-613 (1990).
40. M. van Egmond *et al.*, FcαRI-positive liver Kupffer cells: Reappraisal of the function of immunoglobulin A in immunity. *Nature Medicine* **6**, 680-685 (2000).
41. K. Qian *et al.*, Functional expression of IgA receptor FcαRI on human platelets. *Journal of Leukocyte Biology* **84**, 1492-1500 (2008).
42. C. R. Maliszewski, C. J. March, M. A. Schoenborn, S. Gimpel, L. Shen, Expression cloning of a human Fc receptor for IgA. *Journal of Experimental Medicine* **172**, 1665-1672 (1990).
43. M. A. Kerr, The structure and function of human IgA. *Biochemical Journal* **271**, 285-296 (1990).
44. F.-E. Johansen, R. Braathen, P. Brandtzaeg, The J Chain Is Essential for Polymeric Ig Receptor-Mediated Epithelial Transport of IgA. *The Journal of Immunology* **167**, 5185-5192 (2001).
45. R. Braathen, V. Sørensen, P. Brandtzaeg, I. Sandlie, F.-E. Johansen, The Carboxyl-terminal Domains of IgA and IgM Direct Isotype-specific Polymerization and Interaction with the Polymeric Immunoglobulin Receptor *. *Journal of Biological Chemistry* **277**, 42755-42762 (2002).
46. M. J. Lewis, R. J. Pleass, M. R. Batten, J. D. Atkin, J. M. Woof, Structural Requirements for the Interaction of Human IgA with the Human Polymeric Ig Receptor. *The Journal of Immunology* **175**, 6694-6701 (2005).
47. W.-L. Ling *et al.*, Effect of VH–VL Families in Pertuzumab and Trastuzumab Recombinant Production, Her2 and FcγIIA Binding. *Frontiers in Immunology* **9**, (2018).
48. W.-H. Lua *et al.*, Role of the IgE variable heavy chain in FcεRIα and superantigen binding in allergy and immunotherapy. *Journal of Allergy and Clinical Immunology* **144**, 514-523.e515 (2019).
49. W.-H. Lua *et al.*, The effects of Antibody Engineering CH and CL in Trastuzumab and Pertuzumab recombinant models: Impact on antibody production and antigen-binding. *Scientific Reports* **8**, 718 (2018).
50. C. T.-T. Su, W.-H. Lua, W.-L. Ling, S. K.-E. Gan, Allosteric Effects between the Antibody Constant and Variable Regions: A Study of IgA Fc Mutations on Antigen Binding. *Antibodies* **7**, 20 (2018).
51. W.-H. Lua *et al.*, Discovery of a novel splice variant of FcαR (CD89) unravels sequence segments necessary for efficient secretion: A story of bad signal peptides and good ones that nevertheless do not make it. *Cell Cycle* **16**, 457-467 (2017).
52. S.-X. Phua, W.-H. Lua, S. K.-E. Gan, Role of FcαR EC2 region in extracellular membrane localization. *Cell Cycle* **17**, 669-670 (2018).
53. W.-L. Ling, W.-H. Lua, S. K.-E. Gan, Sagacity in antibody humanization for therapeutics, diagnostics and research purposes: considerations of antibody elements and their roles. *Antibody Therapeutics* **3**, 71-79 (2020).
54. T. Azegami, Y. Yuki, H. Kiyono, Challenges in mucosal vaccines for the control of infectious diseases. *International Immunology* **26**, 517-528 (2014).
55. E. C. Lavelle, R. W. Ward, Mucosal vaccines — fortifying the frontiers. *Nature Reviews Immunology*, (2021).
56. Schrodinger, LLC. (2015).
57. G. C. P. van Zundert *et al.*, The HADDOCK2.2 Web Server: User-Friendly Integrative Modeling of Biomolecular Complexes. *Journal of Molecular Biology* **428**, 720-725 (2016).

58. W.-L. Ling *et al.*, Essentially Leading Antibody Production: An Investigation of Amino Acids, Myeloma, and Natural V-Region Signal Peptides in Producing Pertuzumab and Trastuzumab Variants. *Frontiers in Immunology* **11**, (2020).
59. C. T.-T. Su *et al.*, Molecular Insights of Nickel Binding to Therapeutic Antibodies as a Possible New Antibody Superantigen. *Frontiers in Immunology* **12**, (2021).
60. L. R. Minces, R. K. Shields, K. Sheridan, K. S. Ho, F. P. Silveira, Peptostreptococcus infective endocarditis and bacteremia. Analysis of cases at a tertiary medical center and review of the literature. *Anaerobe* **16**, 327-330 (2010).
61. A. Genovese *et al.*, Immunoglobulin Superantigen Protein L Induces IL-4 and IL-13 Secretion from Human Fc ϵ RI⁺ Cells Through Interaction with the κ Light Chains of IgE. *The Journal of Immunology* **170**, 1854-1861 (2003).
62. V. Patella, V. Casolaro, L. Björck, G. Marone, Protein L. A bacterial Ig-binding protein that activates human basophils and mast cells. *The Journal of Immunology* **145**, 3054-3061 (1990).
63. G. Varricchi *et al.*, Superantigenic Activation of Human Cardiac Mast Cells. *International Journal of Molecular Sciences* **20**, 1828 (2019).
64. M. E. Rosenthal, A. D. Rojzman, E. Frank, Finegoldia magna (formerly Peptostreptococcus magnus): An overlooked etiology for toxic shock syndrome? *Medical Hypotheses* **79**, 138-140 (2012).
65. G. BRUIN, A. FABER, J. BIEWENGA, Binding of Human IgA Fragments to Protein A-Sepharose Studied with an ELISA Method. *Scandinavian Journal of Immunology* **21**, 49-54 (1985).
66. S. R. Fahnestock, P. Alexander, J. Nagle, D. Filpula, Gene for an immunoglobulin-binding protein from a group G streptococcus. *Journal of Bacteriology* **167**, 870-880 (1986).
67. K. J. Reis, E. M. Ayoub, M. D. Boyle, Streptococcal Fc receptors. I. Isolation and partial characterization of the receptor from a group C streptococcus. *The Journal of Immunology* **132**, 3091-3097 (1984).
68. A. GROV, B. MYKLESTAD, P. OEDING, IMMUNOCHEMICAL STUDIES ON ANTIGEN PREPARATIONS FROM STAPHYLOCOCCUS AUREUS. *Acta Pathologica Microbiologica Scandinavica* **61**, 588-596 (1964).
69. F. D. Lowy, Staphylococcus aureus Infections. *New England Journal of Medicine* **339**, 520-532 (1998).
70. M. Chen *et al.*, Clinical applications of detecting IgG, IgM or IgA antibody for the diagnosis of COVID-19: A meta-analysis and systematic review. *International Journal of Infectious Diseases* **104**, 415-422 (2021).
71. E. H. Sasso, G. J. Silverman, M. Mannik, Human IgA and IgG F(ab')₂ that bind to staphylococcal protein A belong to the VHIII subgroup. *The Journal of Immunology* **147**, 1877-1883 (1991).
72. S.-X. Phua, K.-F. Chan, Chinh T.-T. Su, J.-J. Poh, Samuel K.-E. Gan, Perspective: The promises of a holistic view of proteins—impact on antibody engineering and drug discovery. *Bioscience Reports* **39**, (2019).
73. R. E. Snow, C. J. Chapman, A. J. Frew, S. T. Holgate, F. K. Stevenson, Pattern of usage and somatic hypermutation in the VH5 gene segments of a patient with asthma: Implications for IgE. *European Journal of Immunology* **27**, 162-170 (1997).
74. C. Pilette *et al.*, Grass Pollen Immunotherapy Induces an Allergen-Specific IgA2 Antibody Response Associated with Mucosal TGF- β Expression. *The Journal of Immunology* **178**, 4658-4666 (2007).
75. A. K. Gloudemans, B. N. Lambrecht, H. H. Smits, Potential of Immunoglobulin A to Prevent Allergic Asthma. *Clinical and Developmental Immunology* **2013**, 542091 (2013).
76. C. T.-T. Su, W.-L. Ling, W.-H. Lua, J.-J. Poh, S. K.-E. Gan, The role of Antibody V κ Framework 3 region towards Antigen binding: Effects on recombinant production and Protein L binding. *Scientific Reports* **7**, 3766 (2017).

77. S. K.-E. Gan, S. X. Phua, J. Yeo, *Sagacious epitope selection and the things about them that matter in vaccines, and both antibody-based therapeutics and diagnostics, with lessons from virology and oncology.* (2021).
78. F. Samsudin, J. Y. Yeo, S. K.-E. Gan, P. J. Bond, Not all therapeutic antibody isotypes are equal: the case of IgM versus IgG in Pertuzumab and Trastuzumab. *Chemical Science* **11**, 2843-2854 (2020).
79. U. Steffen *et al.*, IgA subclasses have different effector functions associated with distinct glycosylation profiles. *Nature Communications* **11**, 120 (2020).
80. W.-T. Chan, Chandra S. Verma, David P. Lane, Samuel K.-E. Gan, A comparison and optimization of methods and factors affecting the transformation of Escherichia coli. *Bioscience Reports* **33**, (2013).
81. S. Lyskov *et al.*, Serverification of Molecular Modeling Applications: The Rosetta Online Server That Includes Everyone (ROSIE). *PLOS ONE* **8**, e63906 (2013).
82. B. D. Weitzner *et al.*, Modeling and docking of antibody structures with Rosetta. *Nature Protocols* **12**, 401-416 (2017).
83. Y. Ohyama *et al.*, Analysis of O-glycoforms of the IgA1 hinge region by sequential deglycosylation. *Scientific Reports* **10**, 671 (2020).
84. S.-J. Park *et al.*, CHARMM-GUI Glycan Modeler for modeling and simulation of carbohydrates and glycoconjugates. *Glycobiology* **29**, 320-331 (2019).
85. Mark Abraham, Berk Hess, David van der Spoel, E. Lindahl, GROMACS 2019.3 Manual. *Zenodo*, (2019).
86. F. Nadalin, A. Carbone, Protein–protein interaction specificity is captured by contact preferences and interface composition. *Bioinformatics* **34**, 459-468 (2017).

Supplementary

Figure S1: Correlated domain motions of the full-length IgA1 and IgA2 to FcαRI (pdb: 1OW0). Directed motions (shown as arrows) of the domains and the motion magnitude (shown by the arrow length) indicate the long-range effects between the V- and C-regions in the full-length antibody variants that resulted in increased motion magnitudes of the C-region as compared to the truncated C-region in the crystal structure 1OW0. The effect is less pronounced in IgA2s due to the differences of hinge length. Bio3D package (86) was used to perform normal mode analysis on the unbound IgA1 and IgA2 Pertuzumab/Trastuzumab models and the IgA1-Fc alone (using 1OW0). Fluctuations were presented using the first non-trivial modes, i.e., modes 7, for each model.

Figure S2: Different epitopes on Her2 with respect to Trastuzumab (dark and light green for heavy and light chain, respectively) and Pertuzumab (dark and light blue) and their exposures quantified using Solvent Accessible Surface Are (SASA).

Table S1: Reported non-binding and binding of protein G and A respectively to IgA from various product specification sheets. <https://www.abcam.com/kits/antibody-binding-affinities-of-protein-a-protein-g-protein-l-and-jacalin>; <https://www.sigmaaldrich.com/SG/en/technical-documents/technical-article/protein-biology/protein-pulldown/protein-a-g-binding>; <https://www.neb.sg/tools-and-resources/selection-charts/affinity-of-protein-ag-for-igg-types-from-different-species>; <https://www.bio-rad-antibodies.com/binding-affinities.html>; <https://cdn.cytivalifesciences.com/dmm3bwsv3/AssetStream.aspx?mediaformatid=10061&destinationid=10016&assetid=19097>; <https://www.agilent.com/cs/library/applications/5991-6094EN.pdf>

Superantigens Binding Strength to IgA		
Companies	Protein G	Protein A
Abcam	No Binding	Weak Binding
Sigma Aldrich	No Binding	Variable Binding
NEB	No Binding	No Binding

Biorad	No Binding	Weak Binding
Cytiva	No Binding	Variable Binding
Aligent	No Binding	Weak Binding