Structural Insight into the High- and Low-Affinity Alleles of Human and Macaque FcγRIIA (CD32A)

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BACKGROUND

Rhesus macaques (RM), *Macaca mulatta*, are one of the main nonhuman primate models for the testing of vaccines and antibody-based therapeutics. Macaques, like humans, have high and low affinity alleles of FcγRIIA (CD32A) which are expressed on macrophages, dendritic cells and other antigen presenting cells. The polymorphism within FcγRIIA potentially influences the efficacy of vaccine responses by modulating FcγRIIA-mediated phagocytosis, i.e. antibody-dependent cellular phagocytosis (ADCP), and the functionality of IgG subclasses, particularly IgG2. For both species we determined the structures of both receptor variants in complex with IgG1 Fc and the structures of the high-affinity allele with IgG2 Fc. These studies have enabled us to elucidate, both within and between species, the molecular mechanisms underlying the affinity differences and specificity of the FcγRIIA receptor for the IgG subclasses. Understanding these molecular details is paramount for advancing the design of more effective vaccines and antibody-based therapies, particularly in the context of immune responses that rely on FcγRIIA-mediated mechanisms such as ADCP.

METHODS

Human receptors were expressed in HEK 293 cells and purified on IgG sepharose and macaque receptors expressed in CHO cells and purified using nickel magnetic beads. IgG1 Fcs were generated by papain digest of IgGs (human IgG1 and IgG2) or as Fc constructs (macaque IgG1 and IgG2) expressed in HEK 293 cells. IgG subclass binding to FcγRIIA was evaluated by surface plasmon resonance. Complexes were made with an excess of Fc, which was removed by purification by gel filtration, and were then used to grow protein crystals for x-ray structure determination.

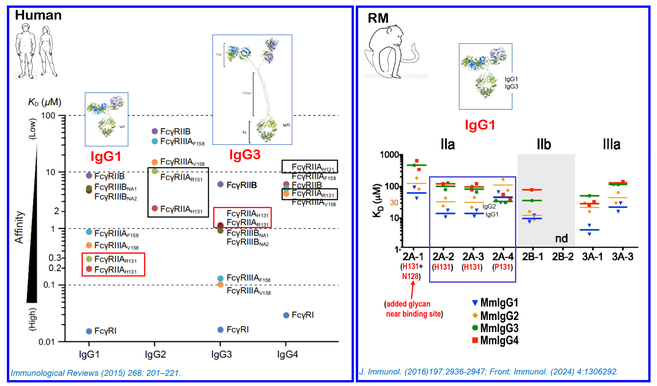


Figure 1. Affinity of human and RM IgG subclasses for species matched Fc receptors. The affinity of both variants of human FcγRIIA to IgG1 and IgG3 are roughly similar[1]. In contrast, in macaques the high affinity H^{131} allele of FcγRIIA has a significantly higher affinity to IgG1 than the low affinity P^{131} allele[2]. Notably, the IgG2 subclass has a much lower affinity to low affinity FcγRIIA allele in both species (R^{131} in humans and P^{131} in macaques).

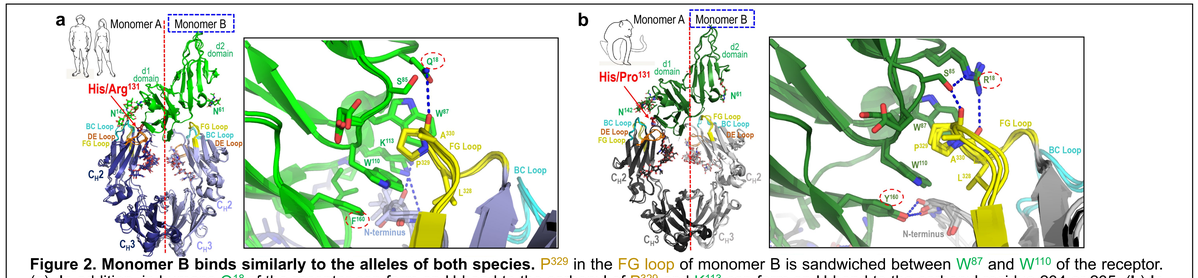


Figure 2. Monomer B binds similarly to the alleles of both species. P^{329} in the FG loop of monomer B is sandwiched between W^{87} and W^{110} of the receptor. (**a**) In addition, in humans Q^{18} of the receptor can form an H-bond to the carbonyl of P^{329} and K^{113} can form an H-bond to the carbonyl residue 234 or 235. (**b**) In macaques R^{18} can form an H-bond to the carbonyl of A^{330} and Y^{160} can form an H-bond to the carbonyl of G^{236}. H-bonds are labeled with dashed blue lines, receptor is green, and Fc and Fc loops are colored as labeled. The polymorphism at residue 131 only interacts with monomer A of the Fc.

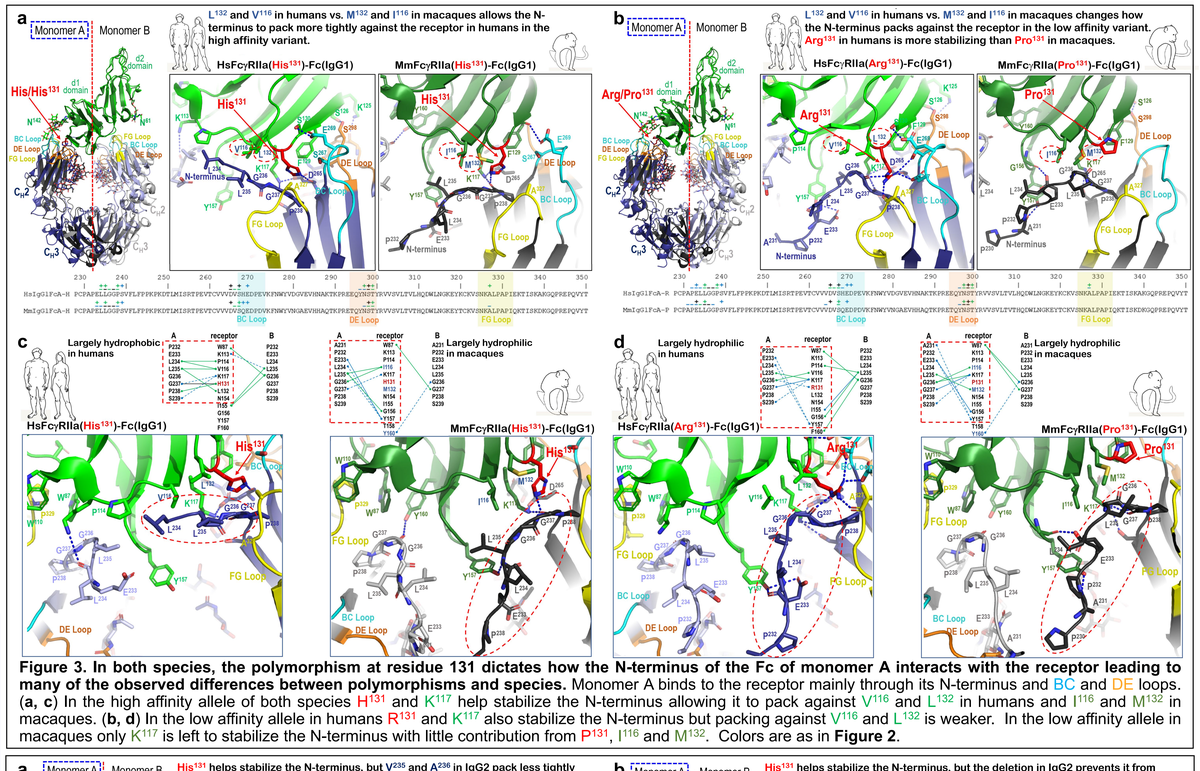


Figure 3. In both species, the polymorphism at residue 131 dictates how the N-terminus of the Fc of monomer A interacts with the receptor leading to many of the observed differences between polymorphisms and species. Monomer A binds to the receptor mainly through its N-terminus and BC and DE loops. (**a, c**) In the high affinity allele of both species H^{131} and K^{117} help stabilize the N-terminus allowing it to pack against V^{116} and L^{132} in humans and I^{116} and M^{132} in macaques. (**b, d**) In the low affinity allele in humans R^{131} and K^{117} also stabilize the N-terminus but packing against V^{116} and L^{132} is weaker. In the low affinity allele in macaques only K^{117} is left to stabilize the N-terminus with little contribution from P^{131}, I^{116} and M^{132}. Colors are as in **Figure 2**.

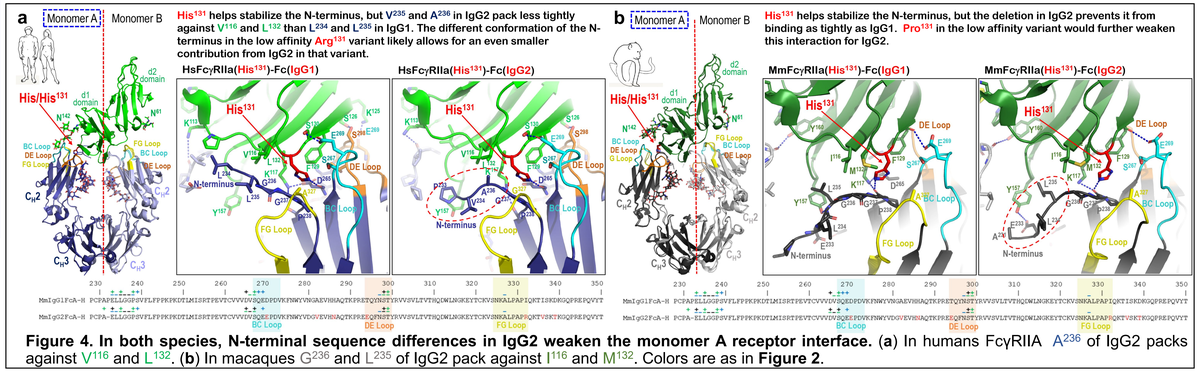


Figure 4. In both species, N-terminal sequence differences in IgG2 weaken the monomer A receptor interface. (**a**) In humans FcγRIIA A^{236} of IgG2 packs against V^{116} and L^{132}. (**b**) In macaques G^{236} and L^{235} of IgG2 pack against I^{116} and M^{132}. Colors are as in **Figure 2**.

RESULTS

We determined the structures of the human hFcγRIIA-H131 and hFcγRIIA-R131 in complex with hIgG1Fc to 2.4 Å and 2.8 Å, the structures of the macaque mFcγRIIA-H131 and mFcγRIIA-P131 with mIgG1Fc to 3.5 Å and 2.7 Å, and the structures of the mFcγRIIA-H131 with mIgG2Fc and of the hFcγRIIA-H131 to hIgG1Fc to 3.2 Å and 2.0 Å, respectively. H^{131} in both species forms an H-bond to monomer A and packs against G^{237} stabilizing the N-terminus similar to His^{134} in FcγRIIIA. P^{131} and R^{131} instead pack against G^{236}; R^{131} also forms an H-bond to the carbonyl of G^{236}. These differences can explain the low affinity allele's reduced affinity for IgG2 in both species.

CONCLUSIONS

In humans, despite noticeable differences in the FcγRIIa complexes for the H^{131} and R^{131} variants, there are smaller differences in affinities across the IgG subclasses except for IgG2. In contrast, in macaques, the P^{131} FcγRIIa variant has a much lower affinity to all IgGs. This may provide a basis for why the H^{131}, R^{131} prevalence in humans is roughly equal, while the P^{131} prevalence in macaques is on the order of 2.5%. Additionally, macaques possess other FcγRIIA polymorphisms that are not present in humans[3,4]. These species-specific variations are critical considerations when assessing FcγRIIa mediated effector functions in macaques in vaccine and antibody infusion trials and translating this to human.

We show how a single amino acid polymorphism changes the FcγRIIA-Fc complex by modulating how the N-terminus of monomer A interacts with FcγR to not only affect affinity but also specificity. Our findings suggest that these structural changes can be leveraged to engineer novel variants of human and RM Abs to modulate ADCP. Moreover, we offer a mechanistic explanation for the observed weak binding of IgG2 to the low-affinity variant of FcγRIIA in both humans and rhesus macaques, highlighting the critical role of specific amino acid interactions in modulating immune responses.

References:

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