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Human IgG lacking effector functions demonstrate lower FcRn-binding and reduced transplacental transport

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ABSTRACT

We have previously generated human IgG1 antibodies that were engineered for reduced binding to the classical Fcγ receptors (FcγRI–III) and C1q, thereby eliminating their destructive effector functions (constant region G1Δnab). In their potential use as blocking agents, favorable binding to the neonatal Fc receptor (FcRn) is important to preserve the long half-life typical of IgG. An ability to cross the placenta, which is also mediated, at least in part, by FcRn is desirable in some indications, such as feto-maternal alloimmune disorders. Here, we show that G1Δnab mutants retain pH-dependent binding to human FcRn but that the amino acid alterations reduce the affinity of the IgG1:FcRn interaction by 2.0-fold and 1.6-fold for the two antibodies investigated. The transport of the modified G1Δnab mutants across monolayers of human cell lines expressing FcRn was approximately 75% of the wild-type, except that no difference was observed with human umbilical vein endothelial cells. G1Δnab mutation also reduced transport in an ex *vivo* placenta model. In conclusion, we demonstrate that, although the G1Δnab mutations are away from the FcRn-binding site, they have long-distance effects, modulating FcRn binding and transcellular transport. Our findings have implications for the design of therapeutic human IgG with tailored effector functions.

1. Introduction

In order to provide an inert IgG Fc region for use in blocking antibodies or fusion proteins where no killing of the target cells should occur, we previously engineered a human IgG1 constant region to reduce its interactions with effector molecules. This was achieved by substituting key motifs of IgG1with residues that are in equivalent positions in the highly homologous but less active constant regions of IgG2 and IgG4. This approach, which substitutes IgG1 residues with equivalents from IgG2 (E233P, L234V, L235A and G236 deleted) (Δb) and from IgG4 (A327G, A330S, P331S) (Δa), minimizes the potential to create new immunogenic epitopes. The locations of these residue changes are illustrated in Fig. 1. The constant region, G1Δab, has been combined with anti-RhD (Fog-1) variable regions to give an antibody that shows minimal binding to FcγRI and III and reduced FcγRII binding (Armour et al., 1999; Armour et al., 2003; Armour et al., 2000). RBC sensitised with the Fog-1 G1Δab do not trigger either ADCC or monocyte activation and the antibody can inhibit activation of these

Abbreviations: FcγR, Fc-gamma receptor; FcRn, neonatal Fc-receptor; FMAIT, fetomaternal alloimmune thrombocytopenia; HPA, human platelet antigens; IVIG, intravenous immunoglobulin; HuVEC, human umbilical vein endothelial cells; JAR, human choriocarcinoma cells

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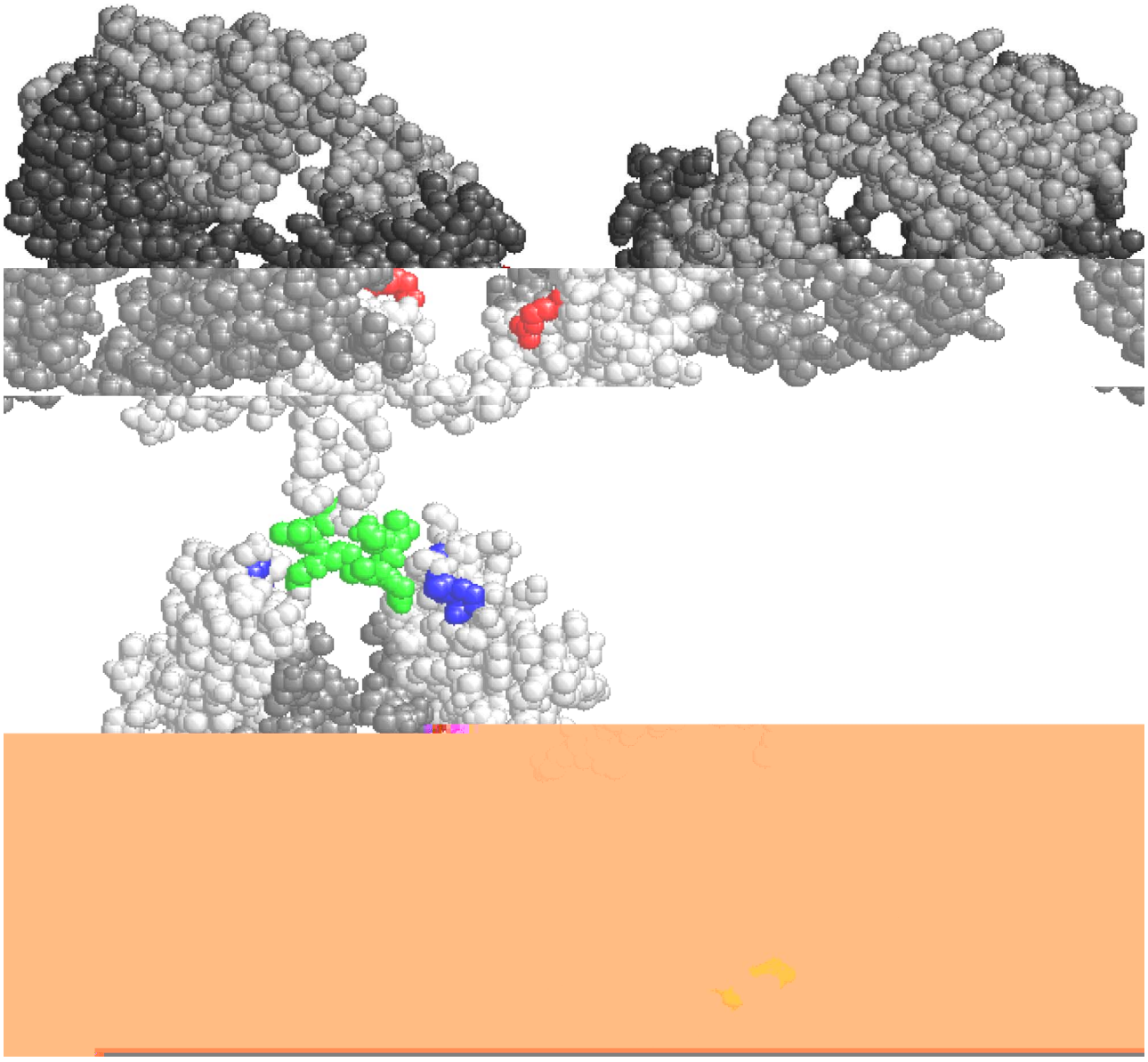

Fig. 1. A structural illustration of human IgG1showing the locations of the residues that were mutated to produce the G1ˆnab constant region. IgG1 heavy chains are shown in light grey with the light chains and Fc-associated carbohydrate in dark grey. The red residues are those altered by the Δn mutation to substitute the IgG1 G1m(1,17) allotypic residues with the corresponding IgG2 residues (K214T, D356E and L358M in the CH1 and CH3). The blue amino acids are changed to IgG4 residues by the Δa mutation (A327G, A330S, P331S in the CH2) and the green residues were substituted with the corresponding amino acids of IgG2 by the Δb mutation (E233P, L234V, L235A, G236 deleted in the lower hinge region of the CH2). The image was generated from the PDB file of an IgG1 model (Clark, 1997) using RasMol V2.7.3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mechanisms by Fog-1 IgG1 or clinical anti-RhD sera (Armour et al., 1999; Armour et al., 2000). In contrast, IgG4 activates monocytes and both IgG2 and IgG4 can mediate ADCC with effector cells from some donors. Thus, the G1Δab constant region is less immunologically activating than native IgG2 or IgG4. Further modification (Δn) has removed one allotypic residue from the CH1 region (K214T) and two allotypic residues from the CH3 region (D356E, L358M) without changing the properties of the constant region (Armour et al., 2006). RBC sensitised with the antibody, Fog-1 G1Δnab, have longer survival *in vivo* in humans than Fog-1 G1-sensitised cells (Armour et al., 2006).

One of our goals, in designing an inert constant region, is to produce a therapeutic antibody for the treatment of fetomaternal alloimmune thrombocytopenia (FMAIT). This condition is caused by the alloimmunisation of pregnant woman against human platelet antigens (HPA) and occurs in approximately 1 in 1500 pregnancies with 85% of these being due to IgG against the HPA-1a antigen on β3 chain of the platelet integrin αIIbβ3 (GPIIbIIIa) (Davoren et al., 2002; van den Akker and Oepkes, 2008). In the most severe cases, intracranial haemorrage causes death or disability. Treatment strategies are currently not ideal. They include increased maternal care, IVIG (with or without steroids) and, less commonly, intrauterine platelet transfusions (van den Akker and Oepkes, 2008). We have a high affinity, highly specific human IgG for HPA-1a (B2) (Griffin and Ouwehand, 1995; Garner et al., 2000) to use as the basis of a therapeutic IgG. Such an antibody could be administered maternally, cross the placenta and bind HPA-1a on fetal platelets, where it could block binding of the maternal cytotoxic anti-HPA-1a IgG1. If the antibody had an inert constant region, the blocking would achieve a reduction in fetal platelet destruction. Indeed, a modified version of the anti-HPA-1a antibody, B2 G1Δnab, reduced monocyte activation in response to platelets sensitised with 18 maternal samples of anti-HPA-1a sera (Ghevaert et al., 2008) by at least 75% and, importantly, did not affect the function of HPA-1a-expressing platelets