CORE
Metadata, citation and similar papers at core.ac.uk
Provided by University of Birmingham Research Portal

UNIVERSITY OF BIRMINGHAM

Research at Birmingham

Enhanced sialylation of a human chimeric IgG1 variant produced in human and rodent cell lines

Mimura, Yusuke; Kelly, Ronan M.; Unwin, Louise; Albrecht, Simone; Jefferis, Royston; Goodall, Margaret; Mizukami, Yoichi; Mimura-kimura, Yuka; Matsumoto, Tsuneo; Ueoka, Hiroshi; Rudd, Pauline M.

DOI:
10.1016/j.jim.2015.11.009

License:
Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

Document Version
Peer reviewed version

Citation for published version (Harvard):
Mimura, Y, Kelly, RM, Unwin, L, Albrecht, S, Jefferis, R, Goodall, M, Mizukami, Y, Mimura-kimura, Y, Matsumoto, T, Ueoka, H & Rudd, PM 2016, 'Enhanced sialylation of a human chimeric IgG1 variant produced in human and rodent cell lines', Journal of Immunological Methods, vol. 428, pp. 30-36. https://doi.org/10.1016/j.jim.2015.11.009

Link to publication on Research at Birmingham portal

Publisher Rights Statement:
Eligibility for repository: checked 08/02/16

General rights
Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

• Users may freely distribute the URL that is used to identify this publication.
• Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
• User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
• Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy
While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Download date: 01. Feb. 2019

Accepted Manuscript

Enhanced sialylation of a human chimeric IgG1 variant produced in human and rodent cell lines

Yusuke Mimura, Ronan M. Kelly, Louise Unwin, Simone Albrecht, Roy Jefferis, Margaret Goodall, Yoichi Mizukami, Yuka Mimura-Kimura, Tsuneo Matsumoto, Hiroshi Ueoka, Pauline M. Rudd

PII: S0022-1759(15)30064-8
DOI: doi: 10.1016/j.jim.2015.11.009
Reference: JIM 12110

To appear in: *Journal of Immunological Methods*

Received date: 1 September 2015
Revised date: 11 November 2015
Accepted date: 23 November 2015

Please cite this article as: Mimura, Yusuke, Kelly, Ronan M., Unwin, Louise, Albrecht, Simone, Jefferis, Roy, Goodall, Margaret, Mizukami, Yoichi, Mimura-Kimura, Yuka, Matsumoto, Tsuneo, Ueoka, Hiroshi, Rudd, Pauline M., Enhanced sialylation of a human chimeric IgG1 variant produced in human and rodent cell lines, *Journal of Immunological Methods* (2015), doi: 10.1016/j.jim.2015.11.009

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Title:

Enhanced sialylation of a human chimeric IgG1 variant produced in human and rodent cell lines

Authors:

Yusuke Mimura[1,2], Ronan M Kelly[2,3], Louise Unwin[2], Simone Albrecht[2], Roy Jefferis[4], Margaret Goodall[4], Yoichi Mizukami[5], Yuka Mimura-Kimura[1,2], Tsuneo Matsumoto[1], Hiroshi Ueoka[1], Pauline M Rudd[2]

Affiliations:

[1]Department of Clinical Research, NHO Yamaguchi-Ube Medical Center, 685 Higashi-Kiwa, Ube, 755-0241 Japan

[2]NIBRT GlycoScience Group, National Institute for Bioprocessing Research and Training, Mount Merrion, Blackrock, Dublin 4, Ireland

[3]Eli Lilly and Company, Indianapolis, USA

[4]School of Immunity and Infection, College of Medical and Dental Sciences, University of Birmingham, Birmingham, B15 2TT, UK

[5]Center for Gene Research, Yamaguchi University, 1-1-1 Minami-Kogushi, Ube, 755-8505, Japan

Abbreviation:

ABS: *Arthrobacter ureafaciens* sialidase; ADCC: antibody-dependent cellular cytotoxicity; BTG: bovine testis galactosidase; CBG: coffee bean galactosidase; CHO: Chinese hamster ovary; DC-SIGN: dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; GlcNAc: N-acetylglucosamine; HEK293: human embryonic kidney 293; IVIG:

ACCEPTED MANUSCRIPT

intravenous immunoglobulin; NP-HPLC: normal-phase high performance liquid chromatography; NAN1: *Streptococcus pneumoniae* sialidase; NIP: 4-hydroxy-3-iodo-5-nitrophenacetyl

Key words:

Glycosylation, IgG, Intravenous immunoglobulin, Sialic acid, Therapeutic antibody

***Corresponding author:**

Yusuke Mimura, M.D., Ph.D.

Department of Clinical Research, NHO Yamaguchi-Ube Medical Center, 685 Higashi-Kiwa, Ube, 755-0241 Japan

Tel: +81 836 582300

Fax: +81 836 585219

E-mail: mimuray@yamaguchi-hosp.jp

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Abstract

Glycosylation of the IgG-Fc is essential for optimal binding and activation of Fcγ receptors and the C1q component of complement. However, it has been reported that the effector functions are down-regulated when the Fc glycans terminate in sialic acid residues and that sialylated IgG mediates anti-inflammatory effects of intravenous immunoglobulin (IVIG). Although recombinant IgG is hypo-sialylated, Fc sialylation is shown to be markedly increased when a mouse/human chimeric IgG3 Phe243Ala (F243A) variant is expressed in Chinese hamster ovary (CHO)-K1 cells. Here we investigate whether sialylation is increased in IgG1 F243A when expressed in CHO-K1, mouse myeloma J558L and human embryonic kidney (HEK) 293. Although the sialylation level was 2 - 5% for IgG1 wild type (WT), it was increased to 31%, 10% and 33% for the variant from CHO-K1, J558L and HEK293 cells, respectively. Interestingly, an increased addition of bisecting GlcNAc and α(1-3)-galactose residues to the Fc glycan was observed for HEK293-derived and J558L-derived IgG1 F243A, respectively. Fucosylation of HEK293-derived IgG1 F243A was maintained despite increased bisecting GlcNAc content. Although sialic acid and bisecting GlcNAc residues are reported to have an opposing effect on antibody-dependent cellular cytotoxicity (ADCC), IgG1 F243A showed 7 times lower ADCC activities than IgG1 WT, irrespective of bisecting GlcNAc residue. Thus, highly sialylated, human cell-derived IgG1 F243A with lowered ADCC activity may be of interest for the development of therapeutic antibodies with anti-inflammatory properties as an alternative to IVIG.

ACCEPTED MANUSCRIPT

1. Introduction

Glycosylation of IgG-Fc at Asn297 is essential for optimal activation of Fcγ receptors and complement (Jefferis, 2007; Jefferis, 2009; Mimura et al., 2009). The oligosaccharides of human serum IgG-Fc are heterogeneous, due to variable attachments of outer-arm sugar residues such as fucose, galactose, bisecting GlcNAc and sialic acid to the core heptasaccharide ($GlcNAc_2Man_3GlcNAc_2$). Importantly, outer-arm sugar residues are involved in unique functions of IgG-Fc glycoforms including enhanced ADCC activity of non-fucosylated glycoforms (Shields et al., 2002; Shinkawa et al., 2003). Conversely, Fc sialylation has been reported to down-regulate Fc effector functions, thereby resulting in reduced ADCC activity (Scallon et al., 2007). It has also been shown that sialylated IgG is responsible for anti-inflammatory effects of intravenous immunoglobulin (IVIG) used for treatment of autoimmune disorders such as Kawasaki disease, immune thrombocytopenia and Guillain-Barré syndrome (Kaneko et al., 2006; Nimmerjahn et al., 2007; Anthony et al., 2008; Anthony and Ravetch, 2010). In a mouse model where mice with induced arthritis were treated by transfer of macrophages from human dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN)-transgenic mice, sialylated IgG was shown to engage DC-SIGN, which resulted in the induction of IL-33 that orchestrates anti-inflammatory responses including the induction of IL-4 from basophils and upregulation of inhibitory receptor FcγRIIb on effector macrophages (Anthony et al., 2011). Although the impact of sialylation of IgG on immunosuppression remains unsolved (Guhr et al., 2011; Kasermann et al., 2012; Leontyev et al., 2012; Schwab and Nimmerjahn, 2013; Yu et al., 2013c; Campbell et al., 2014; Othy et al., 2014; Schwab et al., 2014; Washburn et al., 2015), sialylation of IgG has drawn

ACCEPTED MANUSCRIPT

increased attention as a possible mechanism to generate therapeutic antibodies with anti-inflammatory properties.

Currently approved therapeutic IgG antibodies for clinical use are produced in CHO and mouse myeloma NS0 and Sp2/0 cells, resulting in low levels of IgG sialylation (Mimura et al., 2009). The production of fully sialylated IgG remains a challenge although host cells have been glyco-engineered through overexpression of relevant glycosyltransferases or treated with additives known to enhance glycosyltransferase activities. Amino acid substitution of specific residues in the C_H2 domain of the IgG molecule may provide an alternative route to increasing IgG sialylation level. It has been reported that sialylation is substantially increased for a mouse/human chimeric IgG3 antibody in which Phe243 of the C_H2 domain is replaced by Ala (F243A) and, to a lesser extent, for the IgG3 F241A variant (Lund et al., 1996; Mimura et al., 2001b). Differential scanning microcalorimetry analyses of homogeneous IgG-Fc glycoforms have shown that the removal of the GlcNAc and branching mannose residues that interact with Phe243 and Phe241 residues markedly lower the stability of the C_H2 domains of IgG-Fc (Mimura et al., 2000; Mimura et al., 2001a; Mimura et al., 2001c). Nuclear magnetic resonance (NMR) analysis of the F241A and F243A variants has shown that these amino acid replacements increase the glycan mobility (Subedi et al., 2014), probably exposing the glycans for increased processing by the glycosyltransferases. Crystal structures of the Fc F241A variant have revealed increased conformational flexibility of the C_H2 domains (Yu et al., 2013a; Ahmed et al., 2014), which is consistent with the NMR observation. In this study we have focused on the F243A variant because this IgG1 variant may serve as a suitable antibody model to investigate in greater detail as to the mechanism by which sialylated IgG or IVIG mediates anti-inflammatory properties in humans. As IgG N-glycan composition is species-specific, we selected CHO-K1, mouse myeloma J558L and HEK293 for stable expression of the target

ACCEPTED MANUSCRIPT

protein followed by comprehensive glycan analysis. We found considerable variations in glycan profiles from the IgG1 WT and F243A expressed in these cell lines, primarily due to the species-specific glycosylation machinery. We then examined the effectiveness of the various glycoforms as potential anti-inflammatory biotherapeutics by applying our ex vivo cell-based assay.

2. Materials and methods

2.1. Cell lines

Mouse myeloma cell line J558L (BALB/c) that produces λ L chain and the J558L transfectant THG1-24 that produces anti-NIP (4-hydroxy-3-iodo-5-nitrophenacetyl) mouse/human IgG1 were obtained from European Collection of Cell Culture. CHO-K1 and human embryonic kidney (HEK) 293 were provided by the RIKEN BRC (Ibaraki, Japan). J558L, THG1-24 and CHO-K1 were grown in RPMI1640 cell culture media supplemented with 10% fetal calf serum, 2 mM glutamine and 100 μg/ml penicillin/100 U/ml streptomycin (10% RPMI). HEK293 cells were grown in DMEM supplemented with 10% fetal calf serum, 2 mM glutamine and 100 μg/ml penicillin/100 U/ml streptomycin.

2.2. Construction of expression vectors for mouse/human chimeric IgG1 WT and F243A mutant

2.2.1. Expression of IgG1 WT and F243A in mouse J558L cells

Total RNA was isolated with RNeasy micro kit (Qiagen) from the mouse/human chimeric IgG1-producing THG1-24 cells (Bruggemann et al., 1987). The γ1 gene encoding mouse V_H region (GenBank accession no. **J00529**) and human constant region was reverse-transcribed by

ACCEPTED MANUSCRIPT

using Titan One-Tube RT-PCR kit (Roche) with the forward primer containing *Nco* I site, 5'-GAC CAT GGG ATG GAG CTG TAT CAT GCT-3', and the reverse primer with *Not* I site, 5'-GCG GCC GCT CAT TTA CCC GGA GAC AGG GAG -3'. The PCR products of the γ1 chain gene were ligated to pGEM-T vector (Promega). PCR mutagenesis was performed to replace Phe243 with Ala in the C_H2 domain using the Quickchange II Site-Directed Mutagenesis Kit (Stratagene), the γ1 chain cDNA as the template and mutagenic primers 5'-GAC CGT CAG TCT TCC TCG CCC CCC CAA AAC CCA AG-3' (sense) and 5'-CTT GGG TTT TGG GGG GGC GAG GAA GAC TGA CGG TC-3' (antisense). The γ1 chain Phe243Ala (F243A) DNA was released with *Nco* I and *Not* I restriction endonucleases (New England Biolabs), ligated to pTriEx1.1 neo expression vector (Merck Novagen) and transformed into XL1-Blue supercompetent cells (Stratagene) for plasmid propagation (Fig. 1A-i). The pTriEx1.1 neo expression vector encoding the H chain F243A was transfected by using Nucleofector® (Lonza) into L chain-producing J558L myeloma cells. IgG1 F243A-producing stable clones were established by applying the limiting dilution method in the presence of 0.6 mg/ml G418 (Sigma). The J558L transfectants were grown in the bioreactor CELLine CL1000 (INTEGRA Biosciences) in RPMI1640 containing 2% IgG-depleted fetal calf serum (Life Technologies). and purified on a protein G column (GE) (Fig. 1B-i).

2.2.2. Expression of IgG1 WT and F243A in HEK293 cells

Total RNA was isolated from J558L cells with RNeasy micro kit (Qiagen), and the L chain gene was amplified by RT-PCR with the forward primer 5'-ATG GCC TGG ATT TCA CTT ATA CTC-3' and the reverse primer 5'-CTA GGA ACA GTC AGC ACG GGA CAA-3'. The PCR product was ligated to the pGEM-T easy vector (Promega). Expression vectors encoding both the L chain and the H chain WT or F243A were constructed as follows. The L chain gene was

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

amplified by PCR using the forward primer with the *Xho* I site 5'-ATA GGC TAG CCT CGA GCA CCA TGG CCT GGA TTT CAC TTA TA-3' and the reverse primer with the *Mlu* I site 5'-TGC ATG CTC GAC GCG TCT AGG AAC AGT CAG CAC GGG ACA A-3'. The PCR products and the digested pIRES vector (Clontech) with *Xho* I and *Mlu* I (New England Biolabs) were gel-purified and ligated by the InFusion technology (Clontech) (Fig. 1A-ii). By using the pGEM-T vectors encoding the H chain WT or F243A as a template (Section 2.2.1), the H chain WT or F243A gene was amplified by PCR using the forward primer with the *Xba* I site 5'-CCC GGG ATC CTC TAG ACA CCA TGG GAT GGA GCT GTA TCA TG-3' and the reverse primer with the *Not* I site 5'-TAA AGG GAA GCG GCC GCT CAT TTA CCC GGA GAC AGG GAG A-3'. The PCR products and the L chain-encoding pIRES vector digested with *Xba* I and *Not* I were gel-purified and ligated by the InFusion technology (Fig. 1A-ii). The L and H chain-encoding pIRES vector was transfected into HEK293 cells by electroporation using Neon Transfector (Life Technologies). Stable transfectants producing IgG1 WT or F243A were selected in the presence of G418 (0.4 mg/ml) and cloned by the limiting dilution method. The HEK293 transfectants were grown in the bioreactor CELLine AD1000 (INTEGRA Biosciences) in DMEM containing 2% IgG-depleted fetal calf serum (Life Technologies). IgG1 WT and F243A were purified from the supernatants on a protein G column (GE) (Fig. 1B-i).

2.2.3. Expression of IgG1 WT and F243A in CHO-K1 cells

The L and H chain genes with the intervening IRES sequence (Section 2.2.2) were amplified by PCR using the forward primer with the *Hind* III site 5'-GTT TAA ACT TAA GCT TCA CCA TGG CCT GGA TTT CAC TTA TA-3' and the reverse primer with the *Not* I site 5'-TAG ACT CGA GCG GCC GCT CAT TTA CCC GGA GAC AGG GAG A-3'. The PCR products and the pcDNA5/FRT vector (Life Technologies) digested with *Hind* III and *Not* I were gel-purified and

ACCEPTED MANUSCRIPT

ligated with the InFusion technology (Fig. 1A-ii). CHO-K1 cells were engineered to create a Flp-In CHO host cell line with pFRT/lacZeo vector using the Flp-In System (Life Technologies). The pcDNA5/FRT vector encoding IgG1 WT or F243A and the pOG44 vector were co-transfected into the Flp-In CHO cells by electroporation. Stable CHO transfectants were selected in the presence of 0.2 mg/ml hygromycin and cloned by the limiting dilution method. The CHO transfectants were grown in the bioreactor CELLine AD1000 (INTEGRA Biosciences) in RPMI1640 containing 2% IgG-depleted fetal calf serum. The antibodies were purified from the supernatants on a protein G column (Fig. 1B-i). The antigen binding activities of IgG1 WT and F243A expressed in CHO-K1, J558L and HEK293 cells were compared by ELISA, which confirmed a very similar affinity of these antibodies for the NIP hapten (Fig. S1).

2.3. Glycan analysis by HPLC

N-glycan analysis of IgG by normal-phase high performance liquid chromatography (NP-HPLC) has previously been described (Royle et al., 2006). Briefly, affinity purified IgG (10 – 50 μg) was reduced with SDS sample buffer containing 50 mM dithiothreitol at 70 ^{o}C for 10 min, alkylated with 10 mM iodoacetamide after cooling, and separated by SDS-PAGE. Coomassie Blue-stained H chain gel bands were excised, cut into 1 mm^3, followed by alternate washes with acetonitrile and 20 mM $NaHCO_3$ (pH 7.0). The glycans were released from protein in the gel with peptide-N-glycosidase F (Prozyme) at 37 ^{o}C for 18 h, extracted from the gel pieces by repeated sonication steps in water, then acetonitrile, dried under vacuum and fluorescently labeled with 2-aminobenzamide (2-AB) dye. The 2-AB derivatives were prepared, according to the manufacturer's instruction (LudgerTag^{TM} 2-AB Glycan Labeling Kit, Ludger, UK). The glycans were separated from the excess reagents by ascending paper chromatography in acetonitrile using Whatman 3MM Chr paper. The glycans were analyzed by NP-HPLC on a

ACCEPTED MANUSCRIPT

4.6 x 250 mm TSK amide-80 column (Anachem, Luton, UK). The elution times of glycans are expressed in glucose units by reference to a dextran ladder. The glycans were digested with arrays of exoglycosidases including *Arthrobacter ureafaciens* sialidase (ABS, α2-6>3,8, Prozyme, GK80040), *Streptococcus pneumoniae* sialidase (NAN1, α2-3,8, Prozyme, GK80020), coffee bean α-galactosidase (CBG, α1-3,4,6, Prozyme, GKX-5007) and bovine testis β-galactosidase (BTG, β1-3,4, Prozyme, GKX-5013) for the identification of individual monosaccharides and linkages through enzyme specificity. The digested glycans were cleaned up with Micropure-EZ (Millipore, cat # 42530) before HPLC analysis. Peaks from glycan profiles can be allocated via web-based software that accesses our database (Campbell et al., 2008).

2.4. Glycosidase treatment of IgG1 WT and F243A

For truncation of terminal sialic acid residues, purified IgG1 WT or F243A was treated with ABS (0.25 U/ml, Roche) for 18 h at 37 ^{o}C in 50 mM acetate buffer (pH 5.0). ABS-treated IgG was affinity purified using Streptococcal protein G Sepharose 4B (GE), eluted with 0.1 M glycine-HCl buffer (pH 2.7). Eluates were immediately neutralized with 1 M Tris/HCl (pH 9.0) and dialyzed against PBS (Fig. 1B-ii). Removal of the terminal sialic acid residues was confirmed by analysis of the N-glycan attached to the H chain as described in Section 2.3 and by lectin blotting with *Sambucus Nigra* lectin (Vector Laboratories) for HEK293-derived IgG1 F243A (Fig. 1B-ii).

2.5. ADCC assay

Peripheral blood mononuclear cells (PBMCs) used as effector cells were isolated from heparinized blood with Ficoll-Hypaque at 400 g for 30 min, washed with PBS twice and

ACCEPTED MANUSCRIPT

cultured in 10% RPMI overnight. CHO-K1 cells were suspended in borate-buffered saline (pH 8.0), derivatized with NIP-e-aminocaproyl-OSu (Biosearch Technologies) at 0.1 mg/ml for 30 min by agitating at room temperature, and washed with PBS three times. The NIP-derivatized CHO-K1 cells were resuspended in IMDM (Life Technologies, cat # 12440-053) containing 1% bovine serum albumin at 2 x 10^5/ml and seeded in triplicate in a 96-well plate (50 μl/well). Serially diluted anti-NIP IgG1 WT or F243A (2 x 10^{-4} – 6 μg/ml) were added (50 μl/well). PBMCs resuspended at 2.5 x 10^6/ml in fresh 10% RPMI medium were added to the sensitized target cells (100 μl/well) and incubated for 4 h at 37 °C. One hour before the termination of the incubation 10 μl of 10% Triton-X100 was added to positive control samples to prepare fully lysed controls. The LDH activity from 50 μl of the lysed cell supernatant was measured with the CytoTox96 non-radioactive cytotoxicity assay (Promega). The ADCC data were fitted to a sigmoidal dose-response curve (GraphPad Prism v6). The differences in the ADCC activities between IgG1 WT and various glycoforms of the F243A variant were tested by the extra sum of squares F-test (GraphPad Prism v6). $p < 0.05$ was considered statistically significant.

3. Results

Glycosylation of IgG1 WT and F243A from CHO transfectants

Increased sialylation of the Fc glycans of the F243A variant was first described for IgG3 produced in CHO-K1 transfectants (Lund et al., 1996). We examined if increased sialylation is also observed in the IgG1 subclass by F243A mutation when expressed in stable CHO-K1 transfectants. Although the proportion of sialylated glycoforms was 3.9% for IgG1 WT (Fig. 2A, Table 1), IgG1 F243A exhibited increased levels of sialylated glycoforms (31.0%, Fig. 2B, Table 1). Terminal sialic acid residues were sensitive to digestion with both *Arthrobacter*

ACCEPTED MANUSCRIPT

ureafaciens sialidase (ABS) and *Streptococcus pneumoniae* sialidase (NAN1), indicating that the sialic acids are α(2-3)-linked as predicted (Fig. 2B-ii & iii).

Glycosylation of IgG1WT and F243A from J558L transfectants

Mouse myeloma cells are also of significant industrial importance for the production of therapeutic antibodies. The HPLC profile of IgG1 WT from the mouse J558L transfectant THG1-24 showed minor glycan peaks larger than G2F (Fig. 3A). The glycans larger than G2F were heterogeneous from the IgG1 F243A expressed in stable J558L transfectant compared with those of CHO-derived IgG1 F243A (Fig. 3B-i). Unexpectedly, some of these glycan peaks were not ABS-sensitive (Fig. 3B-ii), in contrast to those of CHO-derived IgG1 (Fig. 2B-ii). A combination of ABS and coffee bean α-galactosidase (CBG) completely digested these glycans to G2(F) (Fig. 3B-ii & iii). This result indicates the presence of α-galactosylated glycans G2FGa1 and G2FGa2 as well as sialylated glycans G2FS1 and G2S2. The ratio of α-galactosylated glycans to sialylated ones was 3:1, suggesting a preference for α-galactosyltransferase activity on the terminal end of the G2(F) glycan in mouse myeloma cell lines.

Glycosylation of IgG1 WT and F243A from HEK293 transfectants

IgG1 WT produced in HEK293 cells also displayed low levels of sialylation compared to the same molecule expressed in the other two rodent cell lines (Fig. 4A-i & ii). In depth analysis of the HPLC profiles identified the glycan with a bisecting GlcNAc residue, one of the structural variations of the human N-glycan, which accounts for 10.1% of the relative percentage of glycans from IgG1 WT. The level of sialylated glycans G2FS1 and G2FS2 was markedly increased for the F243A variant from stable HEK293 transfectant (33.1%, Fig. 4B-i, Table 1). In

ACCEPTED MANUSCRIPT

addition, the proportion of bisecting GlcNAc-containing glycans was also increased for the variant (39.3%, Fig. 4B-ii & iii, Table 1). In contrast, neither the CHO-derived nor the J558L-derived IgG antibodies bore bisected glycan.

ADCC of IgG1 WT and F243A

Sialylated IgG fractions of IVIG or recombinant IgG have been reported to have reduced ADCC activity that is mediated by Fcγ receptor III (FcγRIII) on NK cells (Scallon et al., 2007). ADCC activities of the anti-NIP IgG1 WT and F243A expressed in CHO and HEK293 cells were measured by using NIP-derivatized CHO cells as target cells and peripheral blood mononuclear cells as effector cells. The concentrations of the HEK293-derived antibodies that achieved 20% of cytotoxicity, i.e., half of the maximal wild-type activity, was 0.015 μg/ml and 0.1 μg/ml for WT and F243A, respectively (Fig. 5A). This indicates that the ADCC activity of the F243A variant was reduced 7-fold compared with that of IgG1 WT. To examine if the presence of the sialic acid residue affects ADCC, IgG1 F243A was enzymatically de-sialylated. The ADCC activity of de-sialylated IgG1-F243A was comparable to that of mock-digested IgG1-F243A (Fig. 5A), indicating that the Phe243 residue is implicated in FcγRIII binding, irrespective of the terminal sialic acid. Furthermore, we examined the effect of bisecting GlcNAc residue on ADCC because bisecting GlcNAc has been reported to enhance ADCC (Umana et al., 1999). The ADCC activities of de-sialylated HEK293-derived and CHO-derived IgG1 F243A were compared due to the presence and absence of bisecting GlcNAc, respectively. Interestingly, the ADCC activities of these F243A glycoforms were not distinguishable (Fig. 5B), indicating that the presence of bisecting GlcNAc in the glycan of the F243A variant has a negligible effect on the ability of the molecule to modulate ADCC activity.

ACCEPTED MANUSCRIPT

4. Discussion

Intravenous immunoglobulin (IVIG) is one of the limited treatment options for certain autoimmune disorders. Although the mechanism of action of IVIG has not been fully elucidated, sialylated IgG-Fc has been shown to be involved in the anti-inflammatory properties of IVIG in a mouse model (Anthony et al., 2011). Development of recombinant antibodies with efficient anti-inflammatory properties similar to IVIG would therefore be of interest for therapeutic purposes. However, the Fc glycans of recombinant antibodies produced in CHO cells are severely hypo-sialylated (Fig. 2A) (Mimura et al., 2009). Our attempt to increase the sialylation of IgG1 through the F243A mutation achieved approximately 30% of sialylated glycoforms from stably transfected CHO cells (Fig. 2B). Although glycosylation of CHO-derived IgG is generally human-like, sialic acid linkage differs between CHO and humans in that the CHO-derived IgG is α(2-3)-sialylated whilst the human serum IgG is α(2-6)-sialylated (Takeuchi et al., 1988). Previously the production of α(2-6)-sialylated IgG3 F243A has been reported through overexpression of rat α(2-6)-sialylatransferase in CHO cells (Jassal et al., 2001). Although the sialylation level was slightly increased by the cell engineering, the resulting IgG3 was both α(2-3)- and α(2-6)-sialylated with a ratio of 0.9:1. It has recently been reported that nearly 80% of IgG1 F243A is sialylated when the H and L chains, human β(1-4)-galactosyltransferase and α(2-6)-sialyltransferase were transiently co-expressed in CHO cells (Raymond et al., 2015). Although the study successfully optimized the transient expression of highly sialylated IgG F243A from CHO cells, approximately 20% of glycans of the IgG mutant contained the mixture of α(2-3)- and α(2-6)-linked sialic acids. As biantennary glycans with such mixed sialylation linkages are unnatural, their biological consequence is not

ACCEPTED MANUSCRIPT

predictable.

Mouse myeloma cell lines are commonly used for the production of therapeutic antibodies with a low level of α(2-6)-linked sialic acid attached to the terminal end of the IgG glycan (Mimura et al., 2009). Interestingly, we have shown by using mouse myeloma J558L cells that addition of α-galactose takes precedence over sialylation during IgG1 F243A glycan synthesis (Fig. 3B). The presence of increased α(1-3)-galactosylation as well as α(2-6)-sialylation suggests increased accessibility of the respective enzymes to the glycan as a result of disruption of the interactions between Phe243 residue and the glycan. To our knowledge, the increased α(1-3)-linked galactose content in the Fc glycan of an IgG variant from mouse cells has not previously been described. Although other murine myeloma cell lines need to be tested to generalize this finding, it seems unlikely that mouse cell-derived IgG1 F243A variant is appropriate candidate for therapeutic purposes due to immunogenicity of the α(1-3)-linked galactose epitope in humans (Galili et al., 1993; Chung et al., 2008).

Glycan profiles of recombinant IgG antibodies produced in human cells have not been fully documented. The expression of IgG1 F243A in stably transfected HEK293 cells not only increased terminal sialic acid of the glycan but also increased bisecting GlcNAc content (Fig. 4), again suggesting increased exposure and accessibility of glycans to glycosyltransferases due to the F243A mutation. The lack of effect of the bisecting GlcNAc residues of the F243A variant on ADCC (Fig. 5B) may not be consistent with the previous report about enhanced ADCC activity of IgG with bisecting GlcNAc (Umana et al., 1999; Ferrara et al., 2006). In the N-glycan biosynthesis pathway, the addition of bisecting GlcNAc to the core β-mannose residue prevents the action of α1,6-fucosyltransferase (α1,6-FT), resulting in lack of core fucose

ACCEPTED MANUSCRIPT

(Longmore and Schachter, 1982). Overexpression in antibody-producing cells of N-acetylglucosaminyltransferase III (GnT-III) that catalyzes the addition of bisecting GlcNAc is shown to result in the production of non-fucosylated IgG with increased ADCC activity. Importantly, fucosylation of the HEK293-derived IgG1 F243A was maintained despite increased bisecting GlcNAc content (Fig. 4B-ii, Table 1), indicating that α1,6-FT acts on the Fc glycan before GnT-III does in HEK293 cells. The fucosylation level may depend on the balance of expression between GnT-III and α1,6-FT. With regard to sialylation, it should be possible to obtain higher α(2,6)-sialylation levels of the F243A variant from HEK293 through co-expression of galactosyltransferase and sialyltransferase, as shown with CHO cells (Raymond et al., 2015). Combination of glyco-engineering of human host cell with engineering of Fc amino acid sequence may be an appropriate strategy to produce highly sialylated IgG molecules.

Fc conformational flexibility as well as sialylation has been proposed to account for the anti-inflammatory activity of IgG. Surprisingly, it has recently been reported that the F243A and F241A variants can induce anti-inflammatory cytokine IL-33 production from mouse macrophage expressing DC-SIGN in a similar manner to IVIG and sialylated IgG (Fiebiger et al., 2015). Crystallographic analyses of the F241A Fc (PDB IDs: 4BM7 and 4Q74) have revealed localized destabilization of the protein-glycan interface in the C_H2 domain, indicating a great degree of conformational flexibility compared to native Fc (Yu et al., 2013a; Ahmed et al., 2014). On the other hand, distinct crystal structures of sialylated Fc (PDB IDs: 4BYH, 4Q6Y) suggest heterogeneity of the conformation of the sialylated C_H2 domains (Crispin et al., 2013; Ahmed et al., 2014), which might be related to DC-SIGN engagement. Another key question exists around the IVIG anti-inflammatory mechanism regarding the identification of the human

ACCEPTED MANUSCRIPT

counterpart of the macrophage from the DC-SIGN-transgenic mouse. Although in preliminary studies we pulsed human monocyte-derived mature dendritic cells (DC-SIGN-positive) with IgG1 WT or F243A, IL-33 production was not demonstrated from the dendritic cells (data not shown). The molecular basis for the modulation of autoimmune disorders by IVIG still presents an intriguing question in humans, and the F243A variant could provide a key insight into the mechanism of action of IVIG.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Acknowledgment

This study was supported by JSPS KAKENHI Grant Number 23590578 (Y. M.).

Conflict of interest

None declared.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 1. Analysis of the key features of the N-glycans released from the mouse/human chimeric IgG1 antibodies [a]

Host cell	IgG1	Sialic acid (%)		α-Gal (%)	Term. Gal (%) [b]	Term. GlcNAc (%) [c]	High mannose-type (%)	Bisecting GlcNAc (%)	Core fucose (%)	Predominant glycoform
		S1	S2							
CHO-K1	WT	1.5	2.4	0	70.7	21.9	3.5	0	84	G1F
	F243A	13.4	17.6	0	52.5	11.8	4.7	0	84.1	G2F
J558L	WT	1.1	3.8	6.5	38.6	44.5	5.5	0	87.2	G0F
	F243A	2.7	7.8	28.5	46.7	6.6	7.7	0	69.3	G2F
HEK293	WT	1.4	0.2	0	54.4	42.9	1.1	10.1	88.3	G1F
	F243A	17.5	15.6	0	55.1	6.9	1.6	39.3	90.4	G2F

[a]Glycans were quantitated by measuring peak areas in the HPLC profiles (Figs. 2, 3 and 4).

[b]Glycoforms terminating in galactose residues (G1F, G1FB, G2, G2F and G2FB)

[c]Glycoforms terminating in GlcNAc residues (G0, G0B, G0F and G0FB)

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

References

Ahmed, A.A., Giddens, J., Pincetic, A., Lomino, J.V., Ravetch, J.V., Wang, L.X. and Bjorkman, P.J., 2014, Structural characterization of anti-inflammatory immunoglobulin G Fc proteins. J Mol Biol 426, 3166-79.

Anthony, R.M., Kobayashi, T., Wermeling, F. and Ravetch, J.V., 2011, Intravenous gammaglobulin suppresses inflammation through a novel T(H)2 pathway. Nature 475, 110-3.

Anthony, R.M., Nimmerjahn, F., Ashline, D.J., Reinhold, V.N., Paulson, J.C. and Ravetch, J.V., 2008, Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. Science 320, 373-6.

Anthony, R.M. and Ravetch, J.V., 2010, A novel role for the IgG Fc glycan: the anti-inflammatory activity of sialylated IgG Fcs. J Clin Immunol 30 Suppl 1, S9-14.

Bruggemann, M., Williams, G.T., Bindon, C.I., Clark, M.R., Walker, M.R., Jefferis, R., Waldmann, H. and Neuberger, M.S., 1987, Comparison of the effector functions of human immunoglobulins using a matched set of chimeric antibodies. J Exp Med 166, 1351-61.

Campbell, I.K., Miescher, S., Branch, D.R., Mott, P.J., Lazarus, A.H., Han, D., Maraskovsky, E., Zuercher, A.W., Neschadim, A., Leontyev, D., McKenzie, B.S. and Kasermann, F., 2014, Therapeutic effect of IVIG on inflammatory arthritis in mice is dependent on the Fc portion and independent of sialylation or basophils. J Immunol 192, 5031-8.

Campbell, M.P., Royle, L., Radcliffe, C.M., Dwek, R.A. and Rudd, P.M., 2008, GlycoBase and autoGU: tools for HPLC-based glycan analysis. Bioinformatics 24, 1214-6.

Chung, C.H., Mirakhur, B., Chan, E., Le, Q.T., Berlin, J., Morse, M., Murphy, B.A., Satinover, S.M., Hosen, J., Mauro, D., Slebos, R.J., Zhou, Q., Gold, D., Hatley, T., Hicklin, D.J. and Platts-Mills, T.A., 2008, Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. N Engl J Med 358, 1109-17.

Crispin, M., Yu, X. and Bowden, T.A., 2013, Crystal structure of sialylated IgG Fc: implications for the mechanism of intravenous immunoglobulin therapy. Proc Natl Acad Sci U S A 110, E3544-6.

Ferrara, C., Brunker, P., Suter, T., Moser, S., Puntener, U. and Umana, P., 2006, Modulation of therapeutic antibody effector functions by glycosylation engineering: influence of Golgi enzyme localization domain and co-expression of heterologous beta1, 4-N-acetylglucosaminyltransferase III and Golgi alpha-mannosidase II. Biotechnol

ACCEPTED MANUSCRIPT

Bioeng 93, 851-61.

Fiebiger, B.M., Maamary, J., Pincetic, A. and Ravetch, J.V., 2015, Protection in antibody- and T cell-mediated autoimmune diseases by antiinflammatory IgG Fcs requires type II FcRs. Proc Natl Acad Sci U S A 112, E2385-94.

Galili, U., Anaraki, F., Thall, A., Hill-Black, C. and Radic, M., 1993, One percent of human circulating B lymphocytes are capable of producing the natural anti-Gal antibody. Blood 82, 2485-93.

Guhr, T., Bloem, J., Derksen, N.I., Wuhrer, M., Koenderman, A.H., Aalberse, R.C. and Rispens, T., 2011, Enrichment of sialylated IgG by lectin fractionation does not enhance the efficacy of immunoglobulin G in a murine model of immune thrombocytopenia. PloS one 6, e21246.

Jassal, R., Jenkins, N., Charlwood, J., Camilleri, P., Jefferis, R. and Lund, J., 2001, Sialylation of human IgG-Fc carbohydrate by transfected rat alpha2,6-sialyltransferase. Biochem Biophys Res Commun 286, 243-9.

Jefferis, R., 2007, Antibody therapeutics: isotype and glycoform selection. Expert Opin Biol Ther 7, 1401-13.

Jefferis, R., 2009, Glycosylation as a strategy to improve antibody-based therapeutics. Nature reviews. Drug discovery 8, 226-34.

Kaneko, Y., Nimmerjahn, F. and Ravetch, J.V., 2006, Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science 313, 670-3.

Kasermann, F., Boerema, D.J., Ruegsegger, M., Hofmann, A., Wymann, S., Zuercher, A.W. and Miescher, S., 2012, Analysis and functional consequences of increased Fab-sialylation of intravenous immunoglobulin (IVIG) after lectin fractionation. PloS one 7, e37243.

Leontyev, D., Katsman, Y., Ma, X.Z., Miescher, S., Kasermann, F. and Branch, D.R., 2012, Sialylation-independent mechanism involved in the amelioration of murine immune thrombocytopenia using intravenous gammaglobulin. Transfusion 52, 1799-805.

Longmore, G.D. and Schachter, H., 1982, Product-identification and substrate-specificity studies of the GDP-L-fucose:2-acetamido-2-deoxy-beta-D-glucoside (FUC goes to Asn-linked GlcNAc) 6-alpha-L-fucosyltransferase in a Golgi-rich fraction from porcine liver. Carbohydr Res 100, 365-92.

Lund, J., Takahashi, N., Pound, J.D., Goodall, M. and Jefferis, R., 1996, Multiple interactions of IgG with its core oligosaccharide can modulate recognition by complement and human Fc gamma receptor I and influence the synthesis of its oligosaccharide chains. J Immunol 157, 4963-9.

Mimura, Y., Church, S., Ghirlando, R., Ashton, P.R., Dong, S., Goodall, M., Lund, J. and

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Jefferis, R., 2000, The influence of glycosylation on the thermal stability and effector function expression of human IgG1-Fc: properties of a series of truncated glycoforms. Mol Immunol 37, 697-706.

Mimura, Y., Ghirlando, R., Sondermann, P., Lund, J. and Jefferis, R., 2001a, The molecular specificity of IgG-Fc interactions with Fc gamma receptors. Adv Exp Med Biol 495, 49-53.

Mimura, Y., Jefferis, R., Mimura-Kimura, Y., Abrahams, J. and Rudd, P.M. 2009 Glycosylation of therapeutic IgGs. In: Z. An (Ed.) Therapeutic monoclonal antibodies: From the Bench to the Clinic. Wiley & Sons, Hoboken, p. 67-89.

Mimura, Y., Lund, J., Church, S., Dong, S., Li, J., Goodall, M. and Jefferis, R., 2001b, Butyrate increases production of human chimeric IgG in CHO-K1 cells whilst maintaining function and glycoform profile. Journal of immunological methods 247, 205-16.

Mimura, Y., Sondermann, P., Ghirlando, R., Lund, J., Young, S.P., Goodall, M. and Jefferis, R., 2001c, Role of oligosaccharide residues of IgG1-Fc in Fc gamma RIIb binding. J Biol Chem 276, 45539-47.

Nimmerjahn, F., Anthony, R.M. and Ravetch, J.V., 2007, Agalactosylated IgG antibodies depend on cellular Fc receptors for in vivo activity. Proc Natl Acad Sci U S A 104, 8433-7.

Othy, S., Topcu, S., Saha, C., Kothapalli, P., Lacroix-Desmazes, S., Kasermann, F., Miescher, S., Bayry, J. and Kaveri, S.V., 2014, Sialylation may be dispensable for reciprocal modulation of helper T cells by intravenous immunoglobulin. Eur J Immunol 44, 2059-63.

Raymond, C., Robotham, A., Spearman, M., Butler, M., Kelly, J. and Durocher, Y., 2015, Production of alpha2,6-sialylated IgG1 in CHO cells. MAbs 7, 571-83.

Royle, L., Radcliffe, C.M., Dwek, R.A. and Rudd, P.M., 2006, Detailed structural analysis of N-glycans released from glycoproteins in SDS-PAGE gel bands using HPLC combined with exoglycosidase array digestions. Methods in molecular biology 347, 125-43.

Scallon, B.J., Tam, S.H., McCarthy, S.G., Cai, A.N. and Raju, T.S., 2007, Higher levels of sialylated Fc glycans in immunoglobulin G molecules can adversely impact functionality. Mol Immunol 44, 1524-34.

Schwab, I., Mihai, S., Seeling, M., Kasperkiewicz, M., Ludwig, R.J. and Nimmerjahn, F., 2014, Broad requirement for terminal sialic acid residues and FcgammaRIIB for the preventive and therapeutic activity of intravenous immunoglobulins in vivo. Eur J Immunol 44, 1444-53.

ACCEPTED MANUSCRIPT

Schwab, I. and Nimmerjahn, F., 2013, Intravenous immunoglobulin therapy: how does IgG modulate the immune system? Nature reviews. Immunology 13, 176-89.

Shields, R.L., Lai, J., Keck, R., O'Connell, L.Y., Hong, K., Meng, Y.G., Weikert, S.H. and Presta, L.G., 2002, Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity. J Biol Chem 277, 26733-40.

Shinkawa, T., Nakamura, K., Yamane, N., Shoji-Hosaka, E., Kanda, Y., Sakurada, M., Uchida, K., Anazawa, H., Satoh, M., Yamasaki, M., Hanai, N. and Shitara, K., 2003, The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. J Biol Chem 278, 3466-73.

Subedi, G.P., Hanson, Q.M. and Barb, A.W., 2014, Restricted motion of the conserved immunoglobulin G1 N-glycan is essential for efficient FcgammaRIIIa binding. Structure 22, 1478-88.

Takeuchi, M., Takasaki, S., Miyazaki, H., Kato, T., Hoshi, S., Kochibe, N. and Kobata, A., 1988, Comparative study of the asparagine-linked sugar chains of human erythropoietins purified from urine and the culture medium of recombinant Chinese hamster ovary cells. J Biol Chem 263, 3657-63.

Umana, P., Jean-Mairet, J., Moudry, R., Amstutz, H. and Bailey, J.E., 1999, Engineered glycoforms of an antineuroblastoma IgG1 with optimized antibody-dependent cellular cytotoxic activity. Nat Biotechnol 17, 176-80.

Washburn, N., Schwab, I., Ortiz, D., Bhatnagar, N., Lansing, J.C., Medeiros, A., Tyler, S., Mekala, D., Cochran, E., Sarvaiya, H., Garofalo, K., Meccariello, R., Meador, J.W., 3rd, Rutitzky, L., Schultes, B.C., Ling, L., Avery, W., Nimmerjahn, F., Manning, A.M., Kaundinya, G.V. and Bosques, C.J., 2015, Controlled tetra-Fc sialylation of IVIg results in a drug candidate with consistent enhanced anti-inflammatory activity. Proc Natl Acad Sci U S A 112, E1297-306.

Yu, X., Baruah, K., Harvey, D.J., Vasiljevic, S., Alonzi, D.S., Song, B.D., Higgins, M.K., Bowden, T.A., Scanlan, C.N. and Crispin, M., 2013a, Engineering hydrophobic protein-carbohydrate interactions to fine-tune monoclonal antibodies. J Am Chem Soc 135, 9723-32.

Yu, X., Vasiljevic, S., Mitchell, D.A., Crispin, M. and Scanlan, C.N., 2013c, Dissecting the molecular mechanism of IVIg therapy: the interaction between serum IgG and DC-SIGN is independent of antibody glycoform or Fc domain. J Mol Biol 425, 1253-8.

ACCEPTED MANUSCRIPT

Legends

Fig. 1. Production of mouse/human chimeric IgG1 WT and its F243A variant. (A) Schematic representation of the vectors encoding for the human chimeric IgG1 WT and F243A variant in mouse J558L cells (i) and CHO-K1 and HEK293 cells (ii). (B) SDS-PAGE pattern of the IgG proteins produced from CHO-K1, J558L and HEK293 cells (i) and that of sialidase (ABS)-treated H chains of IgG1 WT and F243A stained with Coomassie Brilliant Blue (CBB) (ii, top) and *Sambucus Nigra* (SNA) lectin specific for α(2-6)-linked sialic acid (ii, bottom).

Fig. 2. NP-HPLC analysis of CHO-derived IgG1 WT (A) and F243A (B). Symbols of monosaccharides and lines for showing glycosidic linkages (inset). Note that the F243A variant bears highly sialylated glycans that are *Streptococcus pneumoniae* sialidase (NAN1)-sensitive, i.e., α(2-3)-linked. Glycans are designated by G0, G1 and G2 according to the numbers of terminal galactose followed by the letter F indicating the presence of fucose. [3] and [6] in the G1 glycan codes indicate the attachment of galactose on the 3- and 6-arm, respectively. M6 denotes a high-mannose-type glycan containing six mannose residues.

Fig. 3. NP-HPLC analysis of mouse myeloma J558L-derived IgG1 WT (A) and F243A (B). Note that the F243A variant has increased levels of both sialylated and α-galactosylated glycans. “Ga” designates α(1-3)-linked galactose residue. Peaks marked with asterisk were artifacts.

Fig. 4. NP-HPLC analysis of HEK293-derived IgG1 WT (A) and F243A (B). The letter B in a glycan code indicates the presence of bisecting GlcNAc. Note that the F243A variant exhibits

ACCEPTED MANUSCRIPT

increased levels of the glycans with sialic acid and bisecting GlcNAc residues.

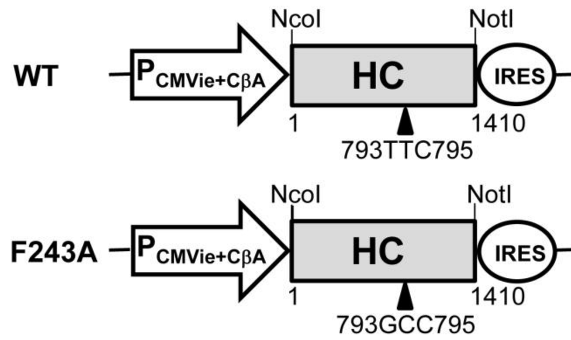
Fig. 5. Comparison of ADCC between IgG1 WT and various F243A glycoforms. (A) Effect of de-sialylation of IgG1 F243A on ADCC. (B) Effect of the presence and absence of bisecting GlcNAc residue in the N-glycans of IgG1 F243A on ADCC. Samples were analyzed in triplicate, and the error bars represent S.E.M. The data were fitted to a sigmoidal dose-response curve. The differences in the ADCC activities between the samples were determined by the extra sum of squares F-test. Note that the presence of sialic acid or bisecting GlcNAc residue(s) in the glycans had no significant effect on the ADCC activity of IgG1 F243A. *$p<0.001$, N.S.: not significant

ACCEPTED MANUSCRIPT

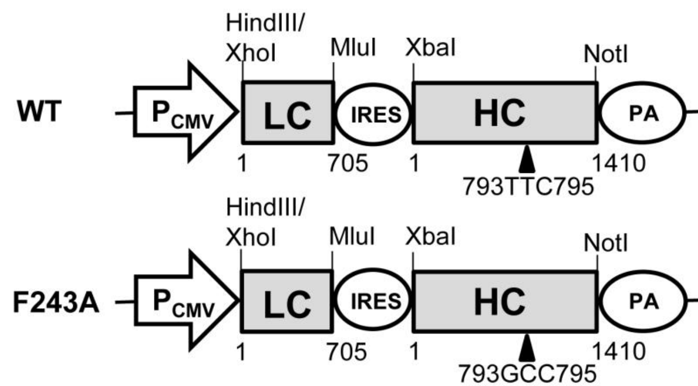
ACCEPTED MANUSCRIPT

A

(i) Vectors for J558L



(ii) Vectors for CHO-K1/HEK293



B

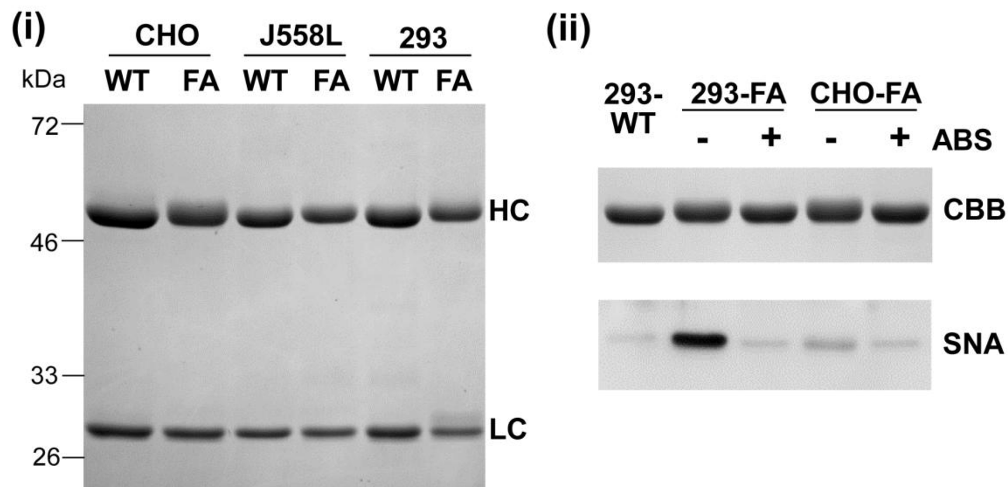


Figure 1

ACCEPTED MANUSCRIPT

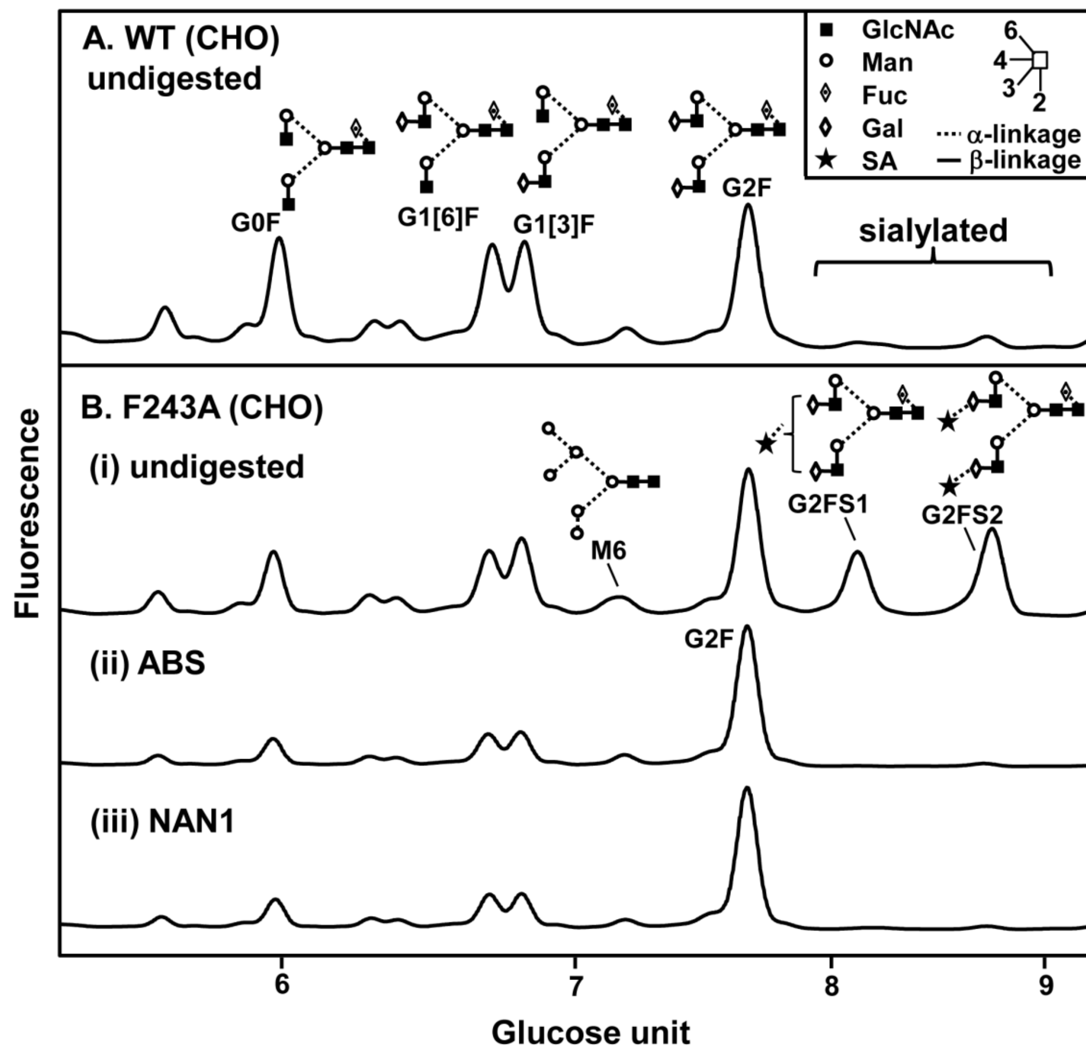


Figure 2

ACCEPTED MANUSCRIPT

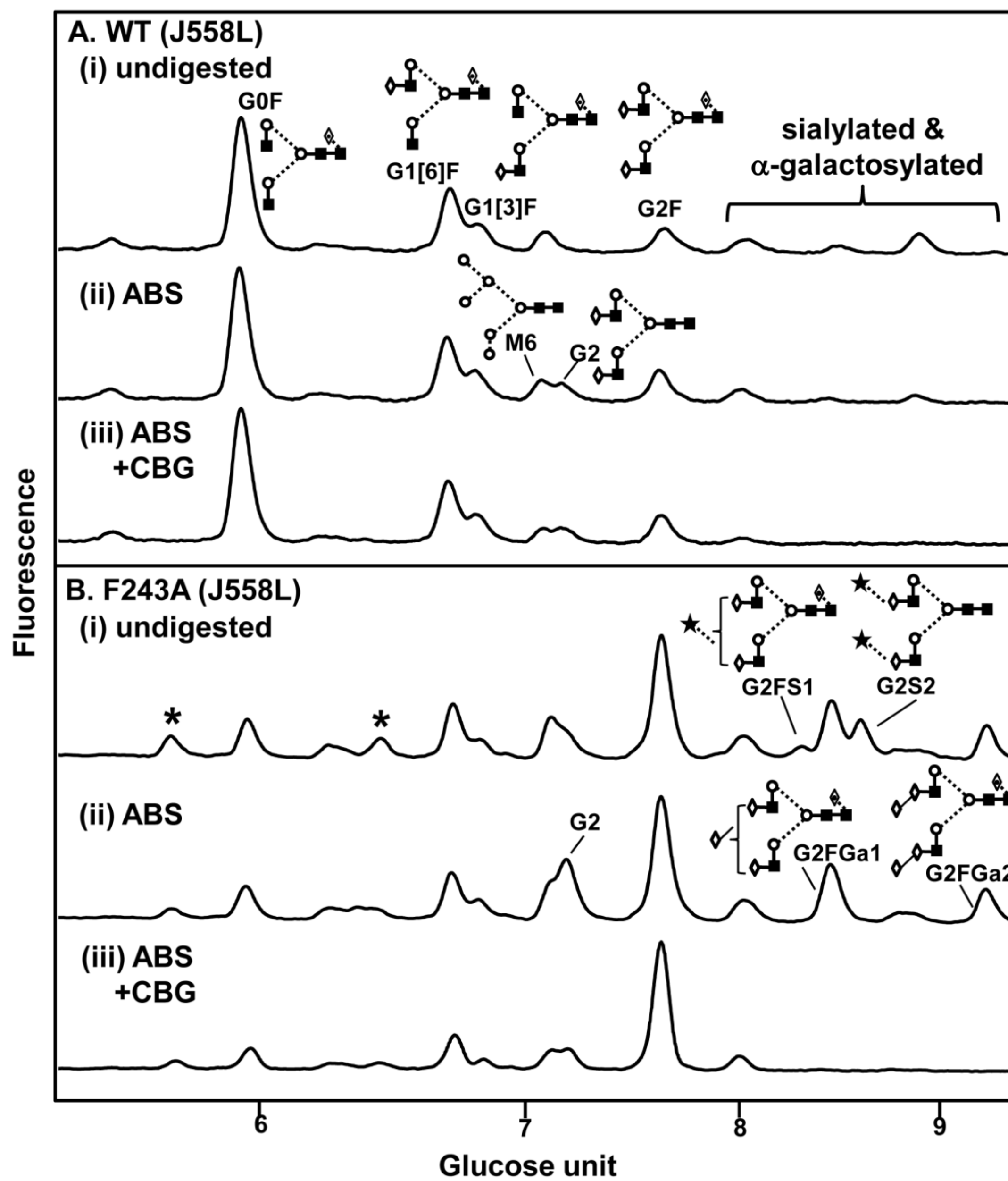


Figure 3

ACCEPTED MANUSCRIPT

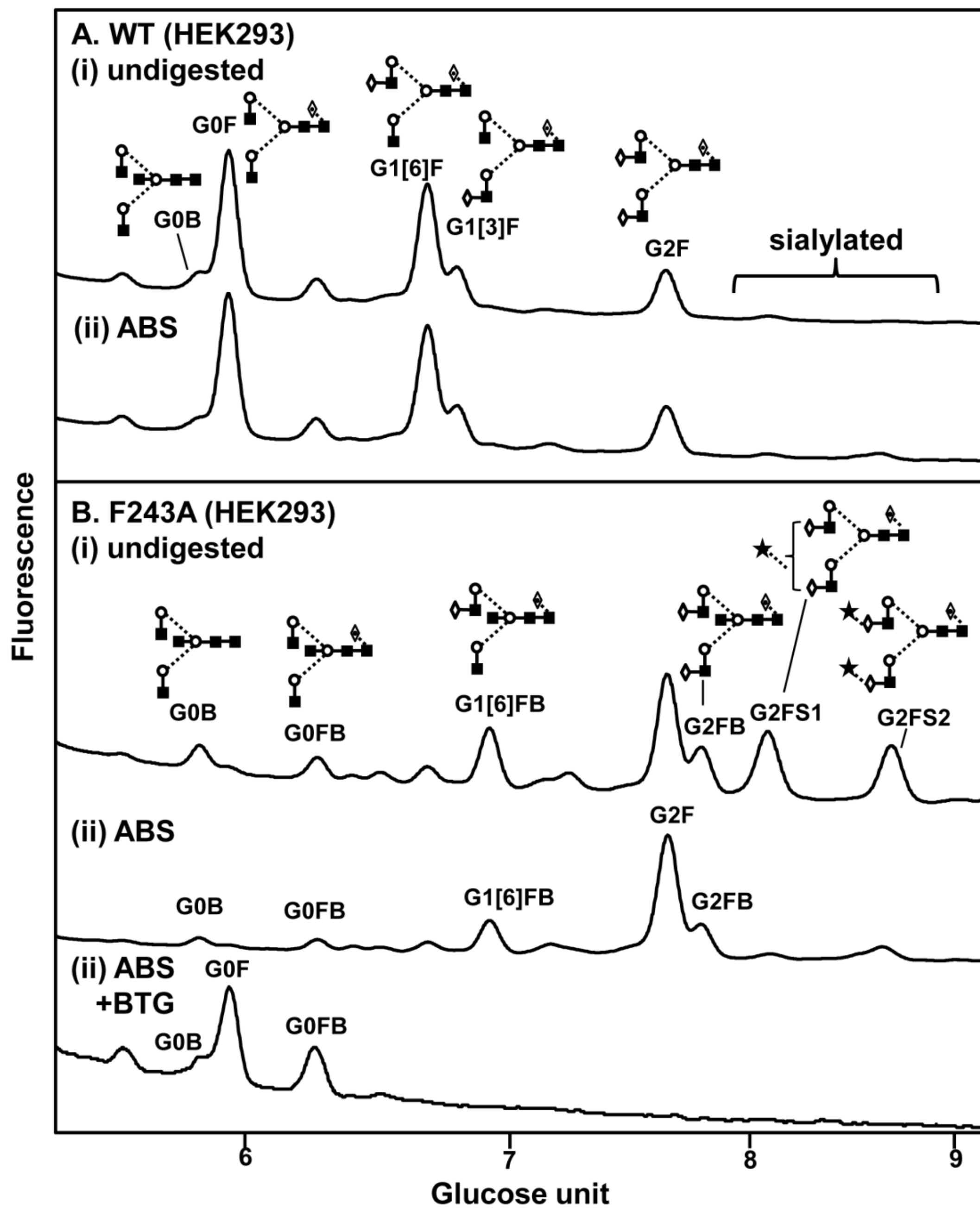


Figure 4

ACCEPTED MANUSCRIPT

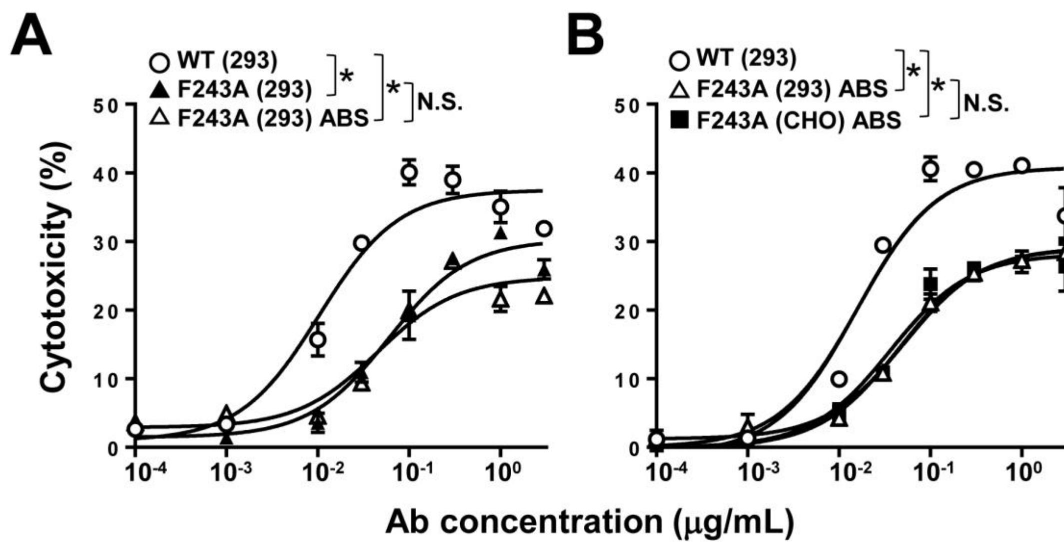


Figure 5

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Enhanced sialylation of a human chimeric IgG1 variant produced in human and rodent cell lines

Yusuke Mimura, *et al.*

Highlights

- Sialylation of IgG1-Fc is enhanced by F243A mutation.
- The glycan structures of IgG1 F243A differ between the species of host cells.
- Low ADCC activity of IgG1 F243A is not influenced by bisecting GlcNAc residue.
- IgG1 F243A can be of interest for development as therapeutics.

ACCEPTED MANUSCRIPT