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CARBOHYDRATE ANTIGEN EXPRESSION AND ANTI-PIG ANTIBODIES IN NEW WORLD CAPUCHIN MONKEYS: RELEVANCE TO STUDIES OF XENOTRANSPLANTATION

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Abstract

Background: Old World nonhuman primates (OWNHPs) are used for preclinical pig-to-NHP studies. However, like pigs, OWNHPs express Neu5Gc, and therefore do not develop natural anti-Neu5Gc antibodies. New World NHPs (NWNHPs) have been reported not to express Neu5Gc. We investigated the potential of NWNHPs in xenotransplantation research.

Methods: We investigated expression of Gal, Neu5Gc, and Sd^a antigens on RBCs and PBMCs from humans, selected OWNHPs, and capuchin monkeys (a NWNHP). Serum anti-Gal and anti-Neu5Gc IgM and IgG levels were measured by ELISA. Binding of primate serum IgM and IgG to pig RBCs was measured by flow cytometry.

Results: (i) Neither humans, OWNHPs, or capuchin monkeys expressed Gal on their RBCs, but capuchins expressed Gal on PBMCs. Humans and capuchins did not express Neu5Gc on either RBCs or PBMCs, but OWNHPs expressed Neu5Gc on both cells. Sd^a was not expressed on any RBCs or PBMCs. (ii) By ELISA, human and OWNHP, but not capuchin, sera showed IgM and

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Disclosure of Conflict of Interest

David Ayares is an employee of Revivacor, Inc., Blacksburg, VA. The other authors declare that they have no conflict of interest.

IgG binding to Gal. Human and capuchin, but not OWNHP, sera demonstrated some binding to Neu5Gc. (iii) Anti-Sd^a IgM/IgG antibodies were detected in OWNHP sera. Knockout of Sd^a on pig RBCs did not significantly reduce human and capuchin antibody binding.

Conclusion: Capuchin monkeys could be surrogates for humans in experiments using RBCs, islets, neuronal cells, etc., from triple-knockout pigs (but may be too small to be used as recipients of pig organ grafts).

Keywords

antibodies; anti-pig; antigens; pig; galactose- α 1,3-galactose; monkey; New World; N-glycolylneuraminic acid; Sd^a; xenotransplantation

INTRODUCTION

Wild-type (WT) pig cells express galactose- α 1,3-galactose (Gal), N-glycolylneuraminic acid (Neu5Gc), and the product of porcine β 1,4-N-acetylgalactosaminyltransferase 2 (β 4GalNT2) indicated as Sd^a (which might be a different product from human β 4GalNT2) [1–3]. As humans do not express any of these three glycans (though many express a human version of Sd^a), they develop antibodies to these xenoantigens [4–6].

Old World nonhuman primates (OWNHPs), e.g., baboons, rhesus macaques, cynomolgus macaques, do not express Gal and so, like humans, produce anti-Gal antibodies. However, OWNHPs express Neu5Gc and so do not make anti-Neu5Gc antibodies [3,7,8]. They are therefore not suitable for studies designed to investigate expression (or lack of expression) of Neu5Gc in pigs. New World NHPs (NWNHPs) have been reported *not* to express Neu5Gc antigens [9], but there have been very few previous studies relating to anti-pig antibodies in these monkeys [10]. The expression of Sd^a, the product of β 4GalNT2, on pig cells also acts as a xenoantigen to humans and OWNHPs [1,4,5]. There is no definitive report whether NWNHPs express Sd^a on their cells.

We have explored the potential role of a NWNHP (capuchin monkey) in xenotransplantation research. Our purpose was to investigate (i) the expression of Gal, Neu5Gc, and Sd^a on pig red blood cells (RBCs) and peripheral blood mononuclear cells (PBMCs), and (ii) the presence of serum anti-pig (especially anti-Neu5Gc) antibodies in the capuchin monkey, and (iii) to compare these results with those from humans and OWNHPs.

These studies are particularly important now that triple-knockout (TKO) pigs are available (i.e., pigs that express none of the three known pig carbohydrate xenoantigens), because OWNHPs (that express Neu5Gc) do not mimic humans in their response to TKO pig cells. Although, in general, NWNHPs may not be large enough for experiments involving organ transplantation, they might prove valuable as surrogates for humans in experiments relating to tissue and cell transplantation (e.g., islets, neuronal cells, corneas, or skin) and pRBC transfusion.

MATERIALS AND METHODS

Human volunteers (who donated blood) gave informed consent, and the study was approved by the University of Pittsburgh Institutional Review Board (#0608179). Animal care accorded with the Guide for the Care and Use of Laboratory Animals prepared by the National Research Council (8th edition, revised 2011), and was conducted in an AAALAC-accredited facility. Protocols were approved by both the University of Pittsburgh (#13082323) and University of Alabama at Birmingham (#20673) Institutional Animal Care and Use Committees.

Sources of primate sera

Sera were obtained from (i) human volunteers of all ABO blood types (22–71y) [3], (ii) OWNHPs (baboons [3–4y] [Oklahoma University Health Sciences Center, Oklahoma City, OK], and rhesus [2–4y] and cynomolgus [2–5y] monkeys [Alpha Genesis, Yemassee, SC]) of all blood types, and (iii) a NWNHP (capuchin [1–25y]) of all blood types (Alpha Genesis).

Sources of pig cells

Pig (p) RBCs and PBMCs were obtained from (i) WT, (ii) α 1,3-galactosyltransferase gene-knockout pigs expressing the human complement-regulatory protein, CD46 (GTKO/CD46 pigs), (iii) GTKO/CD46 pigs with deletion of β 4GalNT2 (GTKO/CD46/ β 4GalNT2KO pigs, i.e., double-knockout pigs), and (iv) a single GTKO/CD46/CMAHKO pig that was found (without further gene modification) not to express Sd^a on its RBCs (though it did express Sd^a on its PBMCs) (designated a GTKO/CD46/CMAHKO [*Mu*] pig, i.e. a TKO pig with regard to RBCs). All pigs were of blood type O (nonA) (Revivicor, Blacksburg, VA). RBCs and PBMCs were isolated as previously described [3,11].

As Neu5Gc can be absorbed from meat in the gastrointestinal tract [12], the genetically-modified pigs were fed a vegetarian diet with no animal-derived protein.

Detection of expression of xenoantigens on selected primate and pig cells by flow cytometry

RBCs and PBMCs from primates and pigs were stained for expression of Gal (by isolectin BSI-B4), Neu5Gc (chicken anti-Neu5Gc mAb), and Sd^a (Dolichos biflorus agglutinin, DBA) [3,13].

Measurement of serum IgM and IgG levels by ELISA

Levels of anti-Gal and anti-Neu5Gc IgM and IgG were measured by ELISA [3,8]. In our preliminary studies, the optimal concentrations of sera were determined as 1:50 for anti-Gal IgM/IgG and 1:10 for anti-Neu5Gc IgM/IgG antibodies (not shown).

Binding of serum IgM and IgG to pRBCs by flow cytometry

Serum antibody binding to pRBCs was measured by flow cytometry using the relative geometric mean (rGM), which was calculated by dividing the geometric mean value for each sample by the negative control, as previously described [3]. The binding of antibodies to

pRBCs was measured by LSR II flow cytometry (BD, San Jose, CA), and analyzed by FlowJo software (Treestar, Ashland, OR).

Statistical analysis

Data are presented as mean and standard deviation (SD) for all variables. The statistical significance of differences was determined by non-parametric Kruskal-Wallis tests followed by Dunn's multiple comparisons test, using GraphPad Prism version 5 (GraphPad Software, San Diego, CA). A p value of 0.05 or less was regarded as significant.

RESULTS

Expression of Gal, Neu5Gc, and Sd^a on primate and pig cells

The expression of these carbohydrates on RBCs (Figures 1A,B) and PBMCs (Figures 1C,D) is summarized in Table 1A.

Neither humans, OWNHPs (baboons, rhesus, and cynomolgus monkeys), nor a NWNHP (capuchin monkey) expressed Gal on their RBCs (Figure 1A), but capuchins did express Gal on PBMCs (Figure 1C). Humans and capuchins did not express Neu5Gc on either RBCs or PBMCs, but OWNHPs expressed Neu5Gc on both cells (Figures 1A,C). Sd^a was not expressed on any primate RBCs or PBMCs (Figures 1A,C).

As anticipated, Gal was expressed on WT pig (p)RBCs and pPBMCs, but not on any GTKO pig cells (Figures 1B,D). Neu5Gc was expressed on all pRBCs and pPBMCs, except on GTKO/CD46/CMAHKO (*Mu*) pig cells (Figures 1B,D). Sd^a was *not* expressed on GTKO/CD46/β4GalNT2KO pRBCs. The single GTKO/CD46/CMAHKO (*Mu*) pig showed no expression of Neu5Gc, and no expression of Sd^a on RBCs, but with moderate expression on PBMCs (Figures 1B,D).

Serum IgM and IgG binding to Gal by ELISA

We detected the highest levels of anti-Gal antibody at a serum dilution of 1:50. Human and OWNHP sera showed IgM and IgG binding to Gal (Figure 2A). There was essentially no binding of capuchin monkey sera to Gal, with significantly lower IgM binding to Gal than human and OWNHP sera ($p < 0.01$ and $p < 0.01$, respectively), and significantly lower IgG binding to Gal than human, baboon, and rhesus monkey sera ($p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively). These data would indicate that capuchin monkeys express Gal, and therefore do *not* make anti-Gal antibody.

Serum IgM and IgG binding to Neu5Gc by ELISA

We detected the highest levels of anti-Neu5Gc antibody at a serum dilution of 1:10. None of the species tested showed more than minimal anti-Neu5Gc IgM (Figure 2B). Human and capuchin sera demonstrated a very low level of IgM binding to Neu5Gc. Human sera showed significantly higher IgG binding to Neu5Gc than sera from other species, including the capuchin monkey (all $p < 0.01$). Capuchin sera showed higher IgG binding to Neu5Gc than OWNHP sera, but this did not reach statistical significance. These data would suggest that humans and capuchin monkeys make anti-Neu5Gc antibody (predominantly IgG).

IgM and IgG binding to WT, GTKO/CD46, GTKO/CD46/ β 4GalNT2KO and GTKO/CD46/CMAHKO (*Mu*) pRBCs by flow cytometry

There was significantly lower binding of IgM and IgG to GTKO/CD46 pRBCs than to WT pRBCs in human and OWNHP sera (except IgG in human sera because the range of binding was large and the number of samples small) (Figure3A). In capuchin monkey sera, no significant differences in IgM and IgG binding were observed, confirming the absence of anti-Gal antibody demonstrated by ELISA.

To investigate whether the expression of Neu5Gc on pRBCs affects primate serum antibody binding, IgM and IgG antibody binding to GTKO/CD46/ β 4GalNT2KO pRBCs was compared to that to GTKO/CD46/CMAHKO (*Mu*) pRBCs (Figure3). Human IgM binding to GTKO/CD46/CMAHKO (*Mu*) pRBCs was significantly reduced. Although lower binding to these cells by capuchin monkey sera was observed, the reduction did not meet statistical significance (Figure 3A). Both human and capuchin serum IgG binding to GTKO/CD46/CMAHKO (*Mu*) pRBCs were reduced compared to binding to GTKO/CD46/ β 4GalNT2KO pRBCs (human: $p < 0.001$, capuchin: $p = 0.31$) (Figure3A).

The results of both ELISA and flow cytometry, therefore, indicated that capuchin monkey sera contain anti-Neu5Gc antibodies, as do human sera. In contrast, OWNHP sera showed higher binding of IgM to GTKO/CD46/CMAHKO (*Mu*) pRBCs than to GTKO/CD46/ β 4GalNT2KO pRBCs. However, when a multiple comparison test was used, statistical significance was not found because the range of data was large and the number of samples small ($n < 14$). Therefore, we further analyzed the data using the combined OWNHP data (including baboons, rhesus, and cynomolgus monkeys, $n = 34$). There was a small but significant increase in antibody binding to GTKO/CD46/CMAHKO [*Mu*] (as TKO) pRBCs compared to GTKO/CD46/ β 4GalNT2KO pRBCs (Figure3B). It is clear that OWNHP serum antibody binding to GTKO/hCD46/CMAHKO[*Mu*], especially IgM binding, is higher than to GTKO/hCD46/ β 4GalNT2KO pRBCs. This indicates that the absence of Gal and Neu5Gc expression exposed one or more new xenoantigens on pig cells which were bound by preformed antibodies in OWNHP sera.

Humans, OWNHPs, and capuchin monkeys (a NWNHP) do not express Sd^a on RBCs or PBMCs (Figure1). However, human sera showed minimal reduced IgM or IgG antibody binding to GTKO/CD46/ β 4GalNT2KO pRBCs than to GTKO/CD46 pRBCs (with no significant difference) (Figure3A). Capuchin sera showed a significant difference in IgM (but not IgG) binding to GTKO/CD46/ β 4GalNT2KO pRBCs than to GTKO/CD46 pRBCs (Figure3A). In contrast, OWNHP sera showed significantly lower binding of antibody to GTKO/CD46/ β 4GalNT2KO pRBCs than to GTKO/CD46 pRBCs (Figure3B).

Experiments were carried out to investigate whether the results obtained using GTKO/CD46/CMAHKO (*Mu*) pRBCs were similar to those obtained using GTKO/CMAHKO/ β 4GalNT2KO (TKO) pRBCs (which were not available to us at the time of the initial study). For this purpose, only human and baboon sera (each $n = 14$) were used because of an insufficient volume of sera from the other NHP species. Human and baboon IgM and IgG antibody binding to TKO pRBCs was not significantly different to binding to GTKO/CD46/CMAHKO (*Mu*) pRBCs (Figure4).

DISCUSSION

NHPs are proving essential surrogates for humans in pig-to-primate organ, tissue, or cell xenotransplantation experiments. The fact that OWNHPs express Neu5Gc renders them less than optimal for experiments involving TKO pig organs and cells [4]. We have investigated the capuchin monkey (a NWNHP) as a potential experimental animal for xenotransplantation, particularly for cell transplants.

Humans have natural antibodies to three antigens expressed on pig cells (Gal, Neu5Gc, Sd^a). We measured (i) expression of Gal, Neu5Gc, and Sd^a on human and selected NHP RBCs and PBMCs, (ii) anti-Gal and anti-Neu5Gc antibodies using sera from humans and selected NHPs, (iii) IgM and IgG binding to pRBCs that did not express Gal or Gal/Sd^a, and (iv) IgM and IgG binding to pRBCs that did not express Gal/Sd^a/Neu5Gc.

Gal

Our data indicate that capuchin monkeys express Gal (on PBMCs, but not on RBCs) whereas humans and OWNHPs do not express Gal on any cells. The capuchin monkey study confirms an observation made previously by Collins et al [10]. Humans and OWNHPs, but not capuchin monkeys, had detectable antibodies that bound Gal, which is consistent with previous reports.

Neu5Gc

The anti-Neu5Gc antibody response will need to be prevented when pig organs or cells are transplanted into human patients [4,7,14–17] unless CMAH gene-knockout (CMAHKO) pigs are the sources of the organs. Blixt [18] demonstrated that some patients developed Neu5Gc-specific antibodies after WT porcine islet transplantation. Scobie [19] reported that patients with burns treated previously with pig skin grafts had significantly higher levels of anti-Neu5Gc IgM or IgG than healthy controls or patients with burns treated without pig skin grafts.

Springer reported that NWNHPs do not express Neu5Gc antigens [9]. *Ex vivo* pig organ perfusion models with human blood have been used to investigate the effect of anti-Neu5Gc antibodies [20, 21]. However, the significance of expression of Neu5Gc in pigs has not been studied in vivo, as all OWNHPs express Neu5Gc.

Our present study indicates that, like humans, capuchin monkeys do not express Neu5Gc, in contrast to OWNHPs. Some capuchin monkeys produce anti-Neu5Gc antibodies (though at low levels), indicating that these monkeys might provide a model in which the effect of Neu5Gc expression on pig cells or tissues (e.g., RBCs, islets, neuronal cells, skin, corneas) could be studied.

One of the limitations of the current study was our method for detecting anti-Neu5Gc antibody by ELISA because anti-Neu5Gc antibodies can bind to multiple forms of Neu5Gc [18], whereas we measured them using an assay with only one synthetic form of Neu5Gc (which was the *only* synthetic Neu5Gc available to us). The Neu5Gc-PAA conjugate that was used for ELISA does not present a sufficiently diverse range of antigens to accurately

measure the amount of anti-Neu5Gc in the serum, and therefore may not give a complete comparison of the antibody levels between humans and capuchins. Although we could positively detect anti-Neu5Gc antibodies in humans and capuchins, but not in other NHPs, our assay probably significantly underestimated the level of anti-Neu5Gc antibody. In future experiments, it will be necessary to use more pertinent methodologies for measuring anti-Neu5Gc antibodies [12,17,22].

To try to confirm that capuchin monkeys make anti-Neu5Gc antibodies, we carried out studies using flow cytometry with Neu5Gc-positive and Neu5Gc-negative pRBCs. Capuchin monkeys had significantly higher levels of anti-Neu5Gc IgG binding to pRBCs than OWNHPs.

Although humans and capuchin monkeys were demonstrated to have anti-Neu5Gc antibodies, these were at much lower levels than anti-Gal antibodies in human serum, although there was considerable variation.

Binding of OWNHP serum antibodies to GTKO/CD46/CMAHKO [Mu] (and to GTKO/CMAHKO/ β 4GalNT2KO [TKO]) pRBCs appeared to be greater than to GTKO/CD46/ β 4GalNT2KO pRBCs. This correlated with the report by Estrada and colleagues [4] who reasoned that this was because knockout of the CMAH gene exposed other antigens against which baboons have natural antibodies. However, Padler-Karavani et al [22] did not observe increased binding after CMAHKO.

Although antibody binding to GTKO/CD46/CMAHKO [Mu] (as TKO) pRBCs was significantly increased in OWNHP (compared to GTKO/CD46/ β 4GalNT2KO), their levels remained low when compared to binding to GTKO/CD46 pRBCs. For studies of TKO pig organ transplantation in OWNHPs, recipients should be selected that have low anti-TKO pig antibodies.

The data suggest that, although the level of natural anti-Neu5Gc antibody in capuchin monkeys is very low, as these monkeys do not express Neu5Gc (and therefore will produce antibodies against this glycan after exposure to it), they may be valuable in demonstrating an elicited antibody response to pRBCs or tissues expressing Neu5Gc, e.g., islets, corneas. OWNHPs could also provide a useful experimental model in several other fields [7,23–27].

Sd^a

Approximately 90% of humans express and/or secrete the Sd^a antigen [28–31], with only a small percentage of human subjects (10%) not expressing Sd^a antigens on their erythrocytes who may therefore possibly develop anti-Sd^a antibodies. In fact, approximately only 1–2% of humans appear to have natural anti-Sd^a antibodies, and these are mainly IgM, not IgG [32].

Therefore, it was initially thought that only a small percentage of individuals would produce antibodies that recognize Sd^a antigens expressed on pRBCs. However, the majority of human serum samples contain antibodies specific for the products of porcine β 4GalNT2 [4, 5]. When expression of Sd^a was deleted from pig cells, a significant reduction of human (and OWNHP) anti-pig antibody binding to those cells was documented. Whether the

products of porcine $\beta 4\text{GalNT2}$ (i.e., porcine Sd^a) are different from those of the human gene (e.g., the structure, distribution, and expression level of Sd^a in cells/tissues) remains uncertain.

Summary

The relative importance of primate anti-pig antibodies in xenotransplantation is summarized in Table 1B. In human and OWNHP sera, anti-Gal antibody would appear to be the most important in relation to pig organ, tissue, or cell xenotransplantation. In humans, anti-Neu5Gc antibody is the next most important, and anti- Sd^a antibody is least important. However, anti- Sd^a antibody is important in OWNHPs. In capuchin monkeys, both anti-Neu5Gc and anti- Sd^a antibodies are important. In this respect, capuchin monkeys have advantages over OWNHPs as a model for xenotransplantation studies relating to TKO pig organ, tissue, or cell transplantation.

Because of the limitations of our study, the value of capuchin monkeys in xenotransplantation research remains uncertain. However, our findings suggest that capuchin monkeys (and possibly other NWNHPs) could be useful surrogates for humans in tissue (e.g., cornea) or cell (e.g., islet, RBCs) xenotransplantation or xenotransfusion experiments from TKO pigs (but may be too small to be used as recipients of pig organ grafts).

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Abbreviations

$\beta 4\text{GalNT2KO}$	β -1,4N-acetylgalactosaminyltransferase 2 gene-knockout
CMAHKO	cytidine monophospho-N-acetylneuraminic acid hydroxylase gene-knockout
Gal	galactose- α 1,3-galactose
GTKO	α 1,3-galactosyltransferase gene-knockout
Neu5Gc	N-glycolylneuraminic acid
NWNHP	New World nonhuman primate
OWNHP	Old World nonhuman primate
PBMCs	peripheral blood mononuclear cells
p	pig
RBCs	red blood cells
TKO	triple-knockout

WT

wild-type

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Figure 1: Expression of Gal, Neu5Gc, and Sd^a on (A, B) RBCs and (C, D) PBMCs from representative humans, baboons, rhesus, cynomolgus, and capuchin monkeys (A, C) and WT, GTKO/CD46, GTKO/CD46/ β 4GalNT2KO and GTKO/CD46/CMAHKO (*Mu*) pigs (B, D)
 The single GTKO/CD46/CMAHKO (*Mu*) pig expressed Sd^a on PBMCs, but not on RBCs.

Figure 2: Anti-Gal IgM/IgG (A) and anti-Neu5Gc IgM/IgG (B) levels in the sera of selected primates

(*Note that the OD scale is different between Figures 2A and 2B)

(A) Serum anti-Gal IgM/IgG levels (at 1:50 dilution)

IgM: The mean anti-Gal IgM serum level in capuchin monkeys (**n=17**) (0.00 ± 0.01) was significantly lower than in humans (**n=11**) (0.52 ± 0.27 , $p < 0.01$), baboons (**n=12**) (0.36 ± 0.12 , $p < 0.01$), rhesus monkeys (**n=4**) (0.55 ± 0.24 , $p < 0.01$), and cynomolgus monkeys (**n=7**) (0.43 ± 0.17 , $p < 0.01$).

IgG: Capuchin sera showed a significantly lower mean anti-Gal IgG level (0.02 ± 0.03) than human (0.93 ± 0.26 , $p < 0.01$), baboons (0.95 ± 0.32 , $p < 0.01$), rhesus monkey (0.66 ± 0.72 , $p < 0.05$), and cynomolgus monkey (0.39 ± 0.34 , $p < 0.05$) sera. (* $p < 0.05$; ** $p < 0.01$)

(B) Serum anti-Neu5Gc IgM/IgG levels (at 1:10 dilution)

IgM: Low or no anti-Neu5Gc IgM was documented in all species tested.

IgG: Human sera (**n=19**) showed significantly higher mean anti-Neu5Gc IgG levels (0.22 ± 0.17) than those in baboon (0.03 ± 0.02 , $p < 0.01$), rhesus (**n=7**) (0.02 ± 0.01 , $p < 0.01$), and cynomolgus monkeys (**n=7**) (0.01 ± 0.01 , $p < 0.01$), and capuchin (**n=17**) (0.06 ± 0.08 , $p < 0.01$) sera. Capuchin sera showed higher mean anti-Neu5Gc IgG levels than in baboon, rhesus and cynomolgus monkey sera.

Figure 3: IgM and IgG binding to WT, GTKO/CD46, GTKO/CD46/β4GalNT2KO and GTKO/CD46/CMAHKO(*Mu*) pRBCs in the sera of human and selected NHPs by flow cytometry

(A) Mean relative GM of IgM (top) and IgG (bottom) comparing binding of human and various NHP sera to WT, GTKO/CD46, GTKO/CD46/β4GalNT2KO, and GTKO/CD46/CMAHKO (*Mu*) pRBCs. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$; **** $p < 0.001$; N.S. = no significant).

(B) Mean relative GM of IgM (left) and IgG (right) comparing binding of combined Old World nonhuman primates (NHPs) sera (baboons, rhesus, and cynomolgus monkeys) to WT, GTKO/CD46, GTKO/CD46/β4GalNT2KO, and GTKO/CD46/CMAHKO (*Mu*) pRBCs.

(* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$; **** $p < 0.001$; N.S. = not significant).

IgM: Combined Old World NHPs sera ($n=34$) showed significantly lower binding of IgM to GTKO/CD46/β4GalNT2KO (relative GM: 1.8 ± 1.1) than to GTKO/CD46/CMAHKO (*Mu*) (3.3 ± 2.1 , $p < 0.05$).

IgG: Combined Old World NHPs sera (n=34) showed significantly lower binding of IgG to GTKO/CD46/ β 4GalNT2KO (relative GM: 1.0 ± 0.1) than to GTKO/CD46/CMAHKO (*Mu*) (relative GM: 1.1 ± 0.2 , $p < 0.01$).

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Figure 4: Human (top: n=14) and baboon (below: n=14) serum IgM and IgG binding to GTKO/CD46/CMAHKO(*Mu*) and TKO pRBCs by flow cytometry
Mean relative GM of IgM and IgG binding demonstrated no significant differences in human or baboon binding to pRBCs from the two different sources.

Table 1A:Expression of Gal, Neu5Gc, and Sd^a on selected primate and pig RBCs and PBMCs

RBCs							
	Human	Old World NHPs	Capuchin	Pigs			
				WT	GTKO/CD46	GTKO/CD46/ β 4GalNT2KO	GTKO/CD46/CMAHKO [<i>Mu</i>]
Gal	-	-	-	+	-	-	-
Neu5Gc	-	+	-	+	+	+	-
Sd ^a	-	-	-	+	+	-	-
PBMCs							
	Human	Old World NHPs	Capuchin	Pigs			
				WT	GTKO/CD46	GTKO/CD46/ β 4GalNT2KO	GTKO/CD46/CMAHKO [<i>Mu</i>]
Gal	-	-	+	+	-	-	-
Neu5Gc	-	+	-	+	+	+	-
Sd ^a	-	-	-	+	+	-	+

Table 1B:

The presence of primate anti-pig antibodies

	Human	Old World NHPs	Capuchin
Anti-Gal antibodies	+	+	-
Anti-Neu5Gc antibodies	+	-	+
Anti-Sd ^a antibodies	+/-	+	+