1    **IgG is differentially and selectively transferred across the placenta in HIV-infected women**

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**Highlights**

- Low peripheral blood CD4 + T cell count and hypergammaglobulinemia are associated with inefficient transplacental IgG transfer in HIV-infected women

- Antigen-specific IgG binding strength to placentally-expressed Fc receptors, but not placental Fc receptor expression levels, mediates selective placental IgG transfer

- Antigen-specific IgG Fc region glycan profiles also contribute to the selective placental IgG transfer of maternal IgG populations in HIV-infected women

**SUMMARY**

The transplacental transfer of maternal IgG to the developing fetus is critical for infant protection against infectious pathogens in the first year of life. However, factors that modulate the transplacental transfer efficiency of maternal IgG that could be harnessed for maternal vaccine design remain largely undefined. HIV-infected women have impaired placental IgG transfer, yet the mechanism underlying this impaired transfer is unknown, presenting an opportunity to explore factors that contribute to the efficiency of placental IgG transfer. We measured the transplacental transfer efficiency of maternal HIV and other pathogen-specific IgG in historical U.S. (n=120) and Malawian (n=47) cohorts of HIV-infected mothers and their HIV-exposed uninfected and HIV-infected infants. We then examined the role of maternal HIV disease progression, infant factors, placental Fc receptor expression, and IgG Fc region subclass and glycan signatures and their association with transplacental transfer efficiency of maternal antigen-specific IgG. We established 3 distinct phenotypes of placental IgG transfer efficiency in HIV-infected women, including: 1) efficient transfer of the majority of antigen-specific IgG populations; 2) generally poor IgG transfer phenotype that was strongly associated with maternal

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64    CD4+ T cell counts, hypergammaglobulinemia, and frequently yielded non-protective levels of

65    vaccine-specific IgG; and 3) variable transfer of IgG across distinct antigen specificities.

66    Interestingly, maternal IgG characteristics, such as binding to placentally expressed Fc receptors

67    FcγRIIa and FcγRIIIa, IgG subclass frequency, and Fc region glycan profiles were associated

68    with placental IgG transfer efficiency. These maternal IgG transplacental transfer determinants

69    were distinct among different antigen-specific IgG populations. Our findings suggest that in

70    HIV-infected women, both maternal disease progression and Fc region characteristics modulate

71    the selective placental transfer of distinct IgG subpopulations, with implications for both the

72    health of HIV-exposed uninfected infants and maternal vaccine design.

73

74    **INTRODUCTION**

75        Protective immunity in the first few months of life is reliant on maternal IgG that is

76    passively transferred across the placenta (Dowling and Levy, 2014; Levy et al., 2013; Palmeira

77    et al., 2012; Zash et al., 2016). This transplacental transfer of protective IgG can be enhanced by

78    maternal vaccination during pregnancy. For example, it is estimated that worldwide incidence

79    rates of neonatal tetanus decreased by 75% from the years 2000 to 2013 due to the wide-scale

80    implementation of maternal tetanus toxoid vaccination during pregnancy (Khan et al., 2015).

81    Furthermore, maternal immunization against influenza has also had a positive impact on infant

82    health, with up to a 63% decrease in influenza infection rates in the first six months of life in

83    infants born to vaccinated mothers compared to those born to unvaccinated women (Zaman et

84    al., 2008). Yet, in 2015, despite the remarkable successes of maternal vaccination, >900,000

85    neonates died from vaccine-preventable respiratory infections worldwide (Liu et al., 2016). As

86    an example, even though the maternal coverage for pertussis vaccination exceeds 80%, neonatal

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87     pertussis rates have increased three-fold over the last three decades in the U.S. alone (Healy et

88     al., 2004). This may be partly due to the fact that maternal passively acquired pertussis-specific

89     IgG can wane to low levels in newborns as early as two months of life, leaving the infant

90     vulnerable to infection (Healy et al., 2004; Nunes et al., 2016). Therefore, there is an urgent need

91     to 1) improve the transplacental IgG transfer efficiency of current maternal vaccines that are

92     routinely administered during pregnancy and 2) develop novel maternal vaccine strategies

93     designed for optimal placental IgG transfer to combat congenital and neonatal infections. On the

94     other hand, minimizing the placental IgG transfer of monoclonal antibody therapies given to

95     pregnant women for their own health is an important goal to improve the safety of this class of

96     therapeutics during gestation.

97        The transplacental transfer of maternal IgG begins in the first trimester of pregnancy and

98     by 20 weeks of gestation, maternal IgG levels in cord blood represent approximately 10% of the

99     maternal blood levels (Malek et al., 1996). In normal pregnancies, by 37-40 weeks of gestation,

100     infant cord blood levels can exceed maternal plasma IgG concentrations, often reaching levels

101     >100% compared to those of their mothers (Kohler and Farr, 1966; Malek et al., 1996; Palmeira

102     et al., 2012; Tatra and Placheta, 1979). Yet in the setting of specific maternal infections, such as

103     HIV, the transplacental transfer of IgG is impaired (Dangor et al., 2015; de Moraes-Pinto et al.,

104     1996; de Moraes-Pinto et al., 1993; de Moraes-Pinto et al., 1998a; de Moraes-Pinto et al., 1998b;

105     Palmeira et al., 2012; Scott et al., 2005). Therefore, HIV-infected women represent a unique

106     population to define factors that modulate the transplacental transfer of maternal IgG to the fetus.

107     Moreover, HIV-exposed uninfected (HEU) infants have up to four-fold higher rates of morbidity

108     and mortality from diarrheal and respiratory infections compared to unexposed infants (Dauby et

109     al., 2016; Locks et al., 2017; Shapiro and Lockman, 2010; Shapiro et al., 2007; Slogrove et al.,

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110    2010; Weinberg et al., 2017). Several factors likely contribute to the high illness and death rates

111    in HEU infants, including the poor transplacental transfer of protective maternal IgG (Adler et

112    al., 2015; Brahmbhatt et al., 2006; Evans et al., 2016; Slogrove et al., 2016). Thus, understanding

113    the mechanisms of impaired transplacental IgG transfer in HIV-infected women could inform

114    strategies to improve the health of HEUs.

115         To reach the fetal circulatory system, maternal IgG must cross distinct placental cell

116    barriers that make up the placental villous tree: the syncytiotrophoblast, the villous stroma, and

117    fetal endothelial cells. The Fc receptor neonatal (FcRn) plays a key role in shuttling maternal IgG

118    across the placenta to the fetal circulatory system (Roopenian and Akilesh, 2007; Simister, 2003;

119    Simister and Mostov, 1989; Simister and Story, 1997). Yet, while syncytiotrophoblast cells

120    express FcRn, neither stromal cells nor fetal endothelial cells express this canonical placental

121    IgG shuttle receptor. Interestingly, other Fcγ receptors are also expressed in placental cells, yet

122    their role in modulating the transplacental transfer of maternal protective IgG is unknown (Fouda

123    et al., 2018; Kristoffersen and Matre, 1996; Martinez et al., 2018; Sedmak et al., 1991; Simister,

124    2003; Simister et al., 1996). Notably, Hofbauer cells located in the villous stroma express FcγRI

125    and FcγRIII, and fetal endothelial cells – the last cell barrier crossed by maternal IgG before

126    reaching the fetal circulatory system – express FcγRII (Kristoffersen et al., 1990; Martinez et al.,

127    2018; Simister, 2003). Gaps in our understanding include how maternal IgG is transferred across

128    this final placental cell barrier in the absence of FcRn, and whether FcγRI, FcγRII, or FcγRIII

129    expression in placental cells contribute to the transplacental transfer of maternal IgG. In addition,

130    IgG characteristics that impact Fc receptor (FcR) interactions, such as IgG subclass and/or Fc

131    region glycans, could play a role in transplacental IgG transfer efficiency. The IgG subclass

132    distribution among different antigen-specific IgG populations is distinct, and previous studies

133 have indicated that this distribution impacts the transplacental transfer efficiency of different

134 antigen-specific IgG populations (Ferrante et al., 1990).

135 In this study, we aimed to explore the mechanism(s) by which transplacental IgG transfer

136 is impaired in HIV-infected women by identifying the determinants of placental IgG transfer

137 through delineation of clinical and antibody characteristics that are associated with poor or

138 efficient placental transfer of antigen-specific IgG populations in 167 HIV-infected pregnant

139 women from the U.S and Malawi. We employed multivariable linear regression modeling to

140 examine the association between transplacental IgG transfer and: 1) maternal HIV disease

141 progression, 2) infant clinical factors, 3) placental factors such as FcR RNA expression, and 4)

142 IgG Fc region characteristics including subclass, glycosylation profiles, and binding strength to

143 placental FcRs. A deeper understanding of factors that modulate the transplacental transfer of

144 maternal protective IgG will be important for improving infant health and extending the window

145 of passively acquired IgG-mediated protection.

146

147 **RESULTS**

148 **Clinical characteristics of U.S. and Malawian mother infant pairs**

149 We assessed the transplacental transfer of HIV-specific and pathogen-specific IgG in two exiting

150 cohorts of HIV infected women: the US-based Women and Infant transmission study (WITS)

151 and the Malawian-based CHAVI009 study. The majority of the n=120 U.S. HIV infected women

152 included in this study did not receive antiretroviral (ART) therapy during pregnancy but, ten of

153 them (8.3%) received azidothymidine (AZT) monotherapy throughout pregnancy and two of

154 them (1.6%) received AZT prophylaxis at delivery. In contrast, 100% of Malawian HIV-infected

155 women received a single dose of nevirapine ART prophylaxis at delivery; and 13 of 47 (28%) of

156     Malawian HIV-infected women with CD4+ T cell counts <250 (cells/mm$^3$) received

157     combination (Stavudine/Lamivudine/Nevirapine) antiretroviral (cART) therapy throughout

158     pregnancy. Overall, HIV-infected U.S. women overall had lower plasma viral load and higher

159     peripheral blood CD4+ T cell counts compared to HIV-infected Malawian women at delivery,

160     despite more Malawian HIV-infected women being initiated on cART during pregnancy (Table

161     1). We measured total plasma IgG levels in U.S. and Malawian women as a surrogate measure of

162     maternal hypergammaglobulinemia, a hallmark feature of advanced HIV-disease progression

163     (Moir et al., 2001). HIV-infected U.S. women had lower total plasma IgG concentrations

164     compared to Malawian women (median plasma IgG concentration levels of 16.3 mg/ml (range

165     2.5 – 123.5 mg/ml) vs 39 mg/ml (range 8.5 – 158.4 mg/ml) in U.S. vs Malawian women), with

166     both populations having higher total plasma IgG concentrations compared to ~10mg/ml that is

167     observed in normal pregnancies (Benster and Wood, 1970), consistent with HIV-associated

168     hypergammaglobulinemia.

169          All 120 infants born to the U.S. HIV-infected women included in this study were

170     uninfected, whereas five out of 47 (10.6%) infants born to Malawian HIV-infected women

171     became infected with HIV *in utero* or peripartum. The U.S. HEU infants had a median

172     gestational age of 40 weeks (range: 30-43 weeks), and Malawian HEU and infected infants had a

173     median gestational age of 39 weeks (range: 33-44 weeks) (Table 1). There were no significant

174     differences in infant birth weight, gestational age, or sex between the two populations.

175

176     **Distinct transplacental IgG transfer efficiency phenotypes in U.S. and Malawian HIV-**

177     **infected women**

178        Previous studies have demonstrated that the transplacental transfer efficiency of maternal

179     vaccine antigen-specific IgG is impaired in the setting of maternal HIV infection (de Moraes-

180     Pinto et al., 1996; de Moraes-Pinto et al., 1993). However, it is unclear if antigen-specific IgG

181     populations are uniformly poorly transferred to the fetus in the setting of maternal HIV infection.

182     We therefore measured HIV-specific IgG antibody levels in U.S. and Malawian paired mother-

183     infant samples against various regions of the envelope glycoprotein including: gp120, variable-

184     loop 3 (V3), variable-loop 1 and 2 (V1V2), and the gp41 membrane-proximal external region

185     (MPER). We also measured antigen-specific IgG antibody levels against common neonatal

186     pathogens, including: influenza hemagglutinin, pertussis toxin, tetanus toxoid, diphtheria toxin,

187     rubella virus capsid, hepatitis B surface antigen, respiratory syncytial virus (RSV) F surface

188     antigen, and *Haemophilus influenzae* type B (Hib) (Figure 1). Both HIV and standard neonatal

189     pathogen-specific IgG responses were detectable at similar frequencies in both U.S. and

190     Malawian HIV-infected women (Table S1).

191        In contrast to previous studies that have only reported a uniformly poor transplacental

192     IgG transfer efficiency among antigen-specific IgG in HIV-infected women(de Moraes-Pinto et

193     al., 1996; de Moraes-Pinto et al., 1998b), we observed three distinct transplacental transfer

194     efficiency phenotypes, including: 1) efficient transplacental IgG transfer efficiency across the

195     majority of measured antigen-specificities, 2) poor transplacental IgG transfer efficiency across

196     the majority of antigen-specificities, and an unexpected phenotype of 3) variable transplacental

197     IgG transfer efficiency across antigen-specific IgG populations (Figure 1).

198        To more quantitatively define transplacental IgG transfer efficiency phenotypes in HIV-

199     infected women from both cohorts across antigen-specific IgG populations, we generated a

200     global transplacental IgG transfer score for each mother-infant pair based on the mean transfer

201   efficiency of the measured IgG populations (Figure 1). Using this scoring system, of 120 U.S.,

202   HIV-infected women, 8.3%, 30.8%, and 60.8% of mother-infant pairs were defined as exhibiting

203   efficient, poor, and variable transplacental IgG transfer, respectively. In contrast, of 47

204   Malawian, HIV-infected women, 0%, 59.5%, and 40.5% mother-infant pairs were defined as

205   exhibiting efficient, poor, and variable transplacental IgG transfer efficiency, respectively. We

206   utilized the global transplacental IgG transfer score for U.S. and Malawian HIV-infected women

207   using multivariable linear regression analyses to define maternal HIV disease progression factors

208   that are associated with placental IgG transfer efficiency. We also generated separate

209   transplacental IgG transfer efficiency scores for three specific IgG populations: gp120, tetanus

210   toxoid, and pertussis toxin-specific IgG. We utilized the antigen-specific IgG transfer score of

211   gp120, tetanus toxoid, and pertussis toxin-specific IgG in multivariable linear regression

212   modeling to define IgG characteristics that are important for transplacental transfer efficiency of

213   these specificities.

214

215   **U.S. and Malawian HIV-infected women with protective IgG concentrations passively**

216   **transfer sub-protective levels of vaccine-specific IgG to their HEU infants**

217   HEU infants are known to be more vulnerable to respiratory and diarrheal diseases and have

218   higher rates of morbidity and mortality compared to HU infants (Afran et al., 2014; Filteau,

219   2009; Slogrove et al., 2016; Zash et al., 2016). We therefore examined the proportion of U.S. and

220   Malawian infants that had cord blood plasma IgG levels below the protective threshold, despite

221   being born to HIV-infected mothers with protective levels of vaccine antigen-specific IgG. We

222   defined protective concentration thresholds as set by the World Health Organization (WHO)

223   (Plotkin, 2010): tetanus toxoid-specific IgG (0.10 IU/ml), rubella-specific IgG (10 IU/ml),

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224     diphtheria toxin-specific IgG (0.10 IU/ml), and Hib-specific IgG (0.15 μg/ml). Of 120 U.S. HIV-

225     infected women, 84%, 36%, 48%, and 22% had plasma concentrations above the protective level

226     threshold for tetanus toxoid-specific IgG, rubella-specific IgG, diphtheria-specific IgG, and Hib-

227     specific IgG, respectively (Figure 2). Yet, due to inefficient IgG transfer, 7%, 16%, 15%, and

228     41% of HEU infants born to these U.S. HIV-infected women with protective IgG levels

229     displayed concentrations below the protective threshold for tetanus toxoid-specific IgG, rubella-

230     specific IgG, diphtheria-specific IgG, and Hib-specific IgG, respectively. Similarly, in 47

231     Malawian HIV-infected women, 87%, 30%, 68%, and 21% had plasma concentrations above the

232     protective level threshold for tetanus toxoid-specific IgG, rubella-specific IgG, diphtheria-

233     specific IgG, and Hib-specific IgG, respectively. Yet, 15%, 71%, 50%, and 70% of infants born

234     to these Malawian HIV-infected women with protective IgG levels had IgG concentrations

235     below the protective threshold for tetanus toxoid, rubella, diphtheria, and Hib, respectively,

236     highlighting the infant health consequences of impaired maternal IgG transfer in HIV-infected

237     women on the infant health.

238

239     **Maternal plasma antigen-specific IgG levels and transplacental IgG transfer efficiency in**

240     **U.S. and Malawian women**

241     To determine whether the antigen-specific IgG concentration in maternal plasma was a primary

242     factor in the distinct transplacental IgG transfer efficiency of both HIV and vaccine antigen-

243     specific IgG, we first examined the association between plasma antigen-specific IgG magnitude

244     and transplacental IgG transfer efficiency (Table 2). In U.S. HIV-infected women, maternal

245     plasma concentrations of HIV V3-specific and RSV surface F antigen-specific IgG was

246     negatively associated with transplacental IgG transfer efficiency (slope: -0.24, p=0.05; slope: -

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247    0.32, p<0.001, respectively). In Malawian HIV-infected women, tetanus toxoid-specific IgG was

248    positively associated with transplacental transfer efficiency (slope: 0.50, p<0.004). Yet, maternal

249    plasma antigen-specific IgG levels were not associated with transplacental transfer efficiency for

250    the majority of HIV and vaccine antigen-specific IgG in both U.S. and Malawian HIV-infected

251    women (Table 2), indicating that factors other than plasma antigen-specific IgG concentration

252    contribute to the efficiency of transplacental IgG transfer.

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254    **Maternal HIV disease progression factors and infant birth characteristics associations with**

255    **transplacental IgG transfer efficiency**

256        It remains unclear how maternal HIV disease progression factors contribute to the poor

257    transplacental IgG transfer observed in HIV-infected women. Maternal CD4+ T cell counts were

258    weakly positively correlated with transplacental IgG transfer efficiency in U.S. HIV-infected

259    women (0.25 p<0.007) but not in Malawian HIV-infected women (0.10 p=0.47) (Figure S1).

260    Interestingly, maternal total plasma IgG was negatively correlated with transplacental IgG

261    transfer efficiency in both U.S. and Malawian HIV-infected women (Figure S1). We therefore

262    sought to examine if maternal peripheral blood CD4+ T cell counts, plasma viral load, and total

263    plasma IgG levels (or hypergammaglobulinemia) contribute to transplacental IgG transfer

264    efficiency in both U.S. and Malawian women in a multivariable linear regression model. We

265    examined maternal clinical characteristics and their relationship to the global transplacental IgG

266    transfer score of each patient by multivariable linear regression. As maternal ART treatment

267    during pregnancy has been associated with improved transplacental IgG transfer (Bosire et al.,

268    2018), we first examined if maternal ART treatment during pregnancy in HIV-infected U.S., and

269    Malawian HIV-infected women was associated with transplacental IgG transfer efficiency.

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270    Maternal ART monotherapy in U.S. HIV-infected women during pregnancy was not correlated

271    with transplacental transfer efficiency (slope: 0.01, p=0.96, FDR=0.96) (Table 3). Similarly,

272    combination ART treatment in Malawian HIV-infected women initiated during pregnancy was

273    not associated with transplacental transfer efficiency (slope: 0.03, p=0.53, FDR=0.85). In this

274    cohort, maternal peripheral blood CD4+ T cell counts were positively associated with

275    transplacental IgG transfer efficiency in U.S. women (slope: 0.19, p<0.002, FDR<0.004), but not

276    in Malawian women (slope: -0.03, p=0.85, FDR=0.85). In contrast, maternal plasma HIV RNA

277    load was not associated with transplacental IgG transfer efficiency in either U.S. (slope: 0.02,

278    p=0.74, FDR=0.93) or Malawian (slope: -0.05, p=0.80, FDR=0.85) women. Yet, maternal total

279    plasma IgG concentrations were negatively associated with transplacental transfer efficiency in

280    both U.S. (slope: -0.37, p=0.001, FDR=0.001) and in Malawian (slope: -0.40, p=0.005,

281    FDR=0.02) HIV-infected women, indicating that hypergammaglobulinemia is associated with

282    poor placental IgG transfer.

283        Infant factors such as gestational age and birth weight have been associated with

284    transplacental IgG transfer efficiency (Palmeira et al., 2012). To assess if these infant factors

285    contributed to the distinct transplacental IgG transfer efficiency phenotypes, we examined infant

286    gestational age, weight, and sex in a multivariable linear regression model in both U.S. HEU and

287    Malawian HEU and HIV-infected infants (Table 3). In these primarily term infant cohorts,

288    neither infant gestational age (U.S. slope: 0.04, p=0.62, FDR=0.62; Malawian slope: 0.24,

289    p=0.03, FDR=0.1), nor weight (U.S. slope: 0.10, p=0.19, FDR=0.55; Malawian slope: -0.33,

290    p=0.05, FDR=0.1), nor sex (U.S. slope: -0.08, p=0.57, FDR=0.62; Malawian slope: 0.08, p=0.74,

291    FDR=0.74) were associated with transplacental transfer efficiency in either maternal-infant

292    cohort.

293

**Maternal co-morbidities in Malawian HIV-infected women and transplacental IgG**

**transfer efficiency**

We next examined maternal co-morbidities that could potentially lead to altered placental IgG

transfer such as maternal proteinuria (a marker of risk of preeclampsia) and syphilis co-infection,

as defined by a maternal rapid plasma reagin (RPR) positive test in the Malawian cohort. These

maternal co-morbidity data were not available for U.S. HIV-infected women. 8.5% of Malawian

HIV-infected women tested positive for the RPR test, whereas 36% tested positive for trace

levels of proteinuria. Interestingly, proteinuria, in addition to total plasma IgG concentration, was

associated with efficient transplacental IgG transfer in Malawian HIV-infected women (slope:

0.55, p=0.01). In contrast, maternal co-infection with syphilis was not significantly associated

with the transplacental transfer efficiency of maternal IgG (slope: -0.79, p=0.06).

305

**Placental FcR expression and transplacental IgG transfer**

To examine the role of placental FcR expression on the transplacental transfer efficiency

of maternal IgG, we performed RNAseq analysis on available placental biopsy tissues only from

Malawian HIV-infected women (n=44), as placental biopsy tissues were not collected in the U.S.

cohort. Maternal placental FcRn, FcγRIIa, FcγIIb, FcγIIc, FcγIIIa, and FcγIIIb mRNA was

detectable in all Malawian HIV-infected women (Figure S2), yet the levels of expression as

defined by $\text{Log}_2$ copies per million (CPM) were variable (Figure S3). We then compared the

levels of placental FcR mRNA expression in HIV-infected women with variable and poor

transplacental transfer of maternal IgG. Placental FcRn, FcγRIIa, FcγIIb, FcγIIc, and FcγIIIa

expression levels strongly correlated with each other, whereas FcγIIIb had lower overall

316      expression levels and a weak correlation with FcRn, FcγRIIa, FcγIIb, FcγIIc, and FcγIIIa

317      expression levels (Figure S2). We did not observe statistically significant differences in FcR

318      expression levels in HIV-infected women with variable and poor transplacental IgG transfer

319      efficiency (Figure S3). We examined Fc receptor expression levels and their relationship to the

320      global transplacental IgG transfer score of each patient by multivariable linear regression. There

321      was no significant association between placental FcR expression levels and transplacental IgG

322      transfer efficiency.

323

324      **Binding of maternal antigen-specific IgG for placental FcRs and transplacental IgG**

325      **transfer**

326      We next assessed the binding of maternal plasma antigen-specific antibodies to placentally-

327      expressed FcRs and their common polymorphic variants in Malawian HIV-infected women

328      (n=47). We measured maternal gp120, tetanus toxoid, and pertussis toxin-specific IgG binding to

329      FcRn, FcγRIIb, FcγRIIIb, the high affinity polymorphic forms FcγRIIa 131H and FcγRIIIa

330      158V, and the low affinity polymorphic variants FcγRIIa 131R and FcγRIIIa 158F, as well as to

331      complement protein C1q (Figure S4). The binding magnitude of gp120 and tetanus toxoid-

332      specific IgG strongly correlated among the measured placental FcRs, whereas the binding

333      magnitude of pertussis toxin-specific IgG to placental FcRs exhibited variable correlations

334      among FcRs (Figure S5). After correcting for predictors of transplacental IgG transfer in HIV-

335      infected Malawian women (total plasma IgG levels and proteinuria), we examined if gp120,

336      tetanus toxin, and pertussis toxin-specific IgG binding to placentally expressed FcRs was

337      associated with transplacental IgG transfer efficiency. We related Fc receptor binding magnitude

338      of gp120, tetanus toxin, and pertussis toxin-specific IgG to their unique antigen-specific

339   transplacental IgG transfer score by multivariable linear regression. Neither maternal gp120 nor

340   pertussis toxin-specific IgG binding to placentally expressed FcRs was associated with their

341   transplacental IgG transfer efficiency (Table 4). In contrast, maternal tetanus toxoid-specific IgG

342   binding to all FcRs positively, albeit weakly, associated with transplacental IgG transfer

343   efficiency (slope: 0.23, p<0.05) (Table 4). Moreover, binding of tetanus toxoid-specific IgG to

344   FcγRIIa 131H (slope: 0.18, p<0.03), FcγRIIa 131R (slope: 0.15, p<0.03), and FcγRIIIa 158F

345   (0.21, p<0.05) were positively associated with transplacental IgG transfer efficiency of this

346   specificity (Table 4), suggesting distinct FcR interaction determinants of placental IgG transfer

347   efficiency for different antigen-specific IgG populations.

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349   **IgG subclass and transplacental IgG transfer efficiency in HIV-infected women with**

350   **variable transplacental IgG transfer efficiency**

351        To further explore IgG Fc characteristics that play a role in antigen-specific IgG

352   transplacental transfer efficiency, we focused on three antigen-specific IgG populations of HIV-

353   infected women defined to have variable transplacental IgG transfer efficiency phenotype:

354   gp120, tetanus toxoid, and pertussis toxin-specific IgG. We measured gp120, tetanus toxoid, and

355   pertussis toxin-specific IgG subclass distribution in both U.S. (n=50) and Malawian (n=16) HIV-

356   infected women with variable transfer of antigen-specific IgG populations (Figure S6). We

357   focused on defining IgG characteristics of gp120-specific IgG because it is generally poorly

358   transferred and on tetanus toxoid and pertussis toxin-specific IgG because they are generally

359   efficiently transferred (Figure S6). The frequency of gp120, tetanus toxoid, and pertussis toxin-

360   specific IgG1 subclass responses was higher than that of the other IgG subclasses in both U.S.

361   and Malawian HIV-infected women. Gp120, tetanus toxoid, and pertussis toxin-specific IgG2

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362    subclass responses were less frequently detected in all antigen-specific populations in U.S.

363    compared to Malawian women (U.S.: gp120-specific 3%, tetanus toxoid-specific 2%, and

364    pertussis toxin-specific 3%; Malawian: gp120-specific 6%, tetanus toxoid-specific 25%, and

365    pertussis toxin-specific 6%, Figure S6). Similarly, U.S. HIV-infected women had relatively

366    lower frequencies of gp120, tetanus toxoid, and pertussis toxin-specific IgG3 subclass responses

367    compared to Malawian women (U.S.: gp120-specific 17%, tetanus toxoid-specific 10%, and

368    pertussis toxin-specific 7%; Malawian: gp120-specific 25%, tetanus toxoid-specific 44%, and

369    pertussis toxin-specific 13%, Figure S6). Lastly, U.S. HIV-infected women also had overall

370    lower frequencies of detectable gp120, tetanus toxoid, and pertussis toxin-specific IgG4 subclass

371    responses compared to Malawian HIV-infected women (U.S.: gp120-specific 2%, tetanus

372    toxoid-specific 27%, and pertussis toxin-specific 2%; Malawian: gp120-specific 19%, tetanus

373    toxoid-specific 81%, and pertussis toxin-specific 0%, Figure S6). In contrast to U.S. HIV-

374    infected women, Malawian HIV-infected women were boosted with a tetanus vaccine during the

375    third trimester of pregnancy, perhaps explaining the overall higher frequencies of tetanus toxoid-

376    specific IgG subclass responses.

377        We then related IgG subclass frequency of gp120, tetanus toxin, and pertussis toxin-

378    specific IgG to their unique antigen-specific transplacental IgG transfer score using multivariable

379    linear regression modelling. We assessed the isolated contribution of antigen-specific IgG

380    subclass distribution to placental IgG transfer efficiency using a multivariable linear regression

381    model that corrected for identified predictors (maternal CD4+ T cell counts and total plasma IgG

382    concentrations) of transplacental IgG transfer. Due to the high collinearity of IgG1 subclass and

383    antigen-specific IgG response frequency, the frequency of antigen-specific IgG responses was

384    included instead of IgG1 subclass in the model (Table 5). Interestingly, in U.S. women, the

16

385    frequency of maternal gp120-specific IgG3 subclass responses (slope: -0.68, p<0.03), the

386    frequency of maternal tetanus toxoid-specific IgG4 subclass responses (slope: -0.52, p=0.04),

387    and the frequency of maternal pertussis-specific IgG responses (slope: -0.33, p=0.02) were

388    negatively associated with their transplacental IgG transfer (Table 5). We did not find that IgG

389    subclass distribution of gp120, tetanus toxoid, or pertussis toxin-specific IgG, to be associated

390    with their transplacental IgG transfer efficiency in Malawian women, likely due to the smaller

391    cohort size.

392

393    **IgG Fc region glycans and transplacental IgG transfer efficiency in HIV-infected women**

394    **with variable transfer efficiency**

395         To examine the potential contribution IgG Fc region glycan profiles in mediating

396    transplacental IgG transfer efficiency, we measured the composition of Fc region glycans in the

397    overall poorly transferred gp120-specific IgG population, and generally efficiently transferred

398    tetanus toxoid and pertussis toxin-specific IgG populations in U.S. HIV-infected women with

399    variable transplacental IgG transfer (n=50) (Figure 3A). We did not observe statistically

400    significant differences of agalactosylated (G0) (Figure 3B) Fc region glycans of gp120, tetanus

401    toxoid, and pertussis toxin-specific IgG. In contrast, we observed statistically significantly

402    different profiles of monogalactosylated (G1) (p<0.0001, Figure 3C), digalactosylated (G2)

403    (p<0.01, Figure 3D), fucosylated (p<0.0001, Figure 3E), bisected (p<0.003, Figure 3F), di-

404    sialylated (p<0.0001, Figure 3G), mono-sialylated (p<0.0001, Figure 3H), and total sialylated

405    (p<0.0001, Figure 3I) Fc region glycans between the isolated IgG for the same specificities.  To

406    determine if specific Fc region glycans were associated with transplacental IgG transfer

407    efficiency, we performed a principal component analysis in which we examined the proportional

17

408    variance of each principal component as it related to the transplacental IgG transfer efficiency.

409    Principal component 1 (composed of G0, G1, G2, mono sialic acid, and total sialic acid, Figure

410    4, Table S2) accounted for 59-64% of the variance of transplacental IgG transfer of gp120,

411    tetanus toxoid, and pertussis toxin-specific IgG (Table S2), whereas principal component 2

412    (composed of fucose, disialic acid, and bisecting, Figure 4, Table S2) accounted for 16-24% of

413    the variance of transplacental IgG transfer for the same specificities.

414         Previous studies reported that a lower frequency of Fc region fucose glycans led to

415    increased binding strength to FcγRIIIa, suggesting that Fc region glycans may modulate binding

416    to Fc receptors (Masuda et al., 2007; Niwa et al., 2004; Okazaki et al., 2004). Interestingly,

417    poorly transferred gp120-specific IgG had higher frequencies of Fc region fucose, whereas

418    efficiency transferred tetanus toxoid and pertussis toxin had overall higher levels of fucose

419    (Figure 3D), suggesting that Fc region glycans may modulate the selective transfer of IgG

420    subpopulations. We therefore related Fc region glycan profiles of gp120, tetanus toxin, and

421    pertussis toxin-specific IgG to their unique antigen-specific transplacental IgG transfer score by

422    multivariable linear regression. We fit a multivariable linear regression model that corrected for

423    identified clinical predictors of transplacental IgG transfer efficiency (maternal CD4+ T cell

424    counts and total plasma IgG concentrations). We found that Fc region glycan signatures of

425    tetanus toxoid or pertussis toxin-specific IgG were not associated with their transplacental IgG

426    transfer efficiency. In contrast, gp120-specific IgG Fc region fucose, bisecting, and disialic acid

427    glycan frequencies (PC2) were weakly positively associated with transplacental IgG transfer

428    efficiency of this specificity (slope: 0.27, p<0.01) (Figure 4A, Figure 4B, and Table 6),

429    suggesting that Fc region fucose, bisected, and disialylated glycans can modulate transplacental

430    IgG transfer efficiency of some but not all antigen-specific IgG subpopulations. We next

18

431    examined the isolated contribution of each individual gp120-specific IgG Fc region glycan as it

432    related to transplacental IgG transfer efficiency. We observed weak yet significant associations

433    with individual gp120-specific Fc region glycans (Table 6). Gp120-specific IgG Fc region fucose

434    and di-sialic acid frequencies were significantly but very weakly associated with transplacental

435    IgG transfer efficiency, suggesting a collective effect of these Fc region glycans on placental IgG

436    transfer (Table 6).

437

438    **Antigen-specific IgG magnitude and transplacental IgG transfer efficiency in HIV-infected**

439    **women with variable transfer efficiency**

440         After again correcting for identified maternal predictors of transplacental IgG transfer

441    efficiency, we reexamined the isolated role of antigen-specific plasma IgG concentrations and

442    their association with transplacental IgG transfer efficiency in U.S. women with variable

443    transplacental IgG transfer efficiency. We examined antigen-specific magnitude of gp120,

444    tetanus toxin, and pertussis toxin-specific IgG by multivariable linear regression using their

445    unique antigen-specific transplacental IgG transfer score. Maternal gp120-specific IgG

446    concentrations were significantly associated with transplacental transfer efficiency in HIV-

447    infected women with variable transplacental IgG transfer efficiency (slope: 0.38, p=0.01) (Table

448    6). In contrast, neither maternal tetanus toxoid-specific IgG concentrations (slope: -0.04, p=0.78)

449    nor maternal pertussis toxin-specific IgG concentrations (slope: -0.03, p=0.89) were associated

450    with their transplacental IgG transfer efficiency in U.S. HIV-infected women with variable

451    transplacental IgG transfer.

452

453 **Combined analysis of IgG characteristics associated with transplacental IgG transfer**

454 **efficiency in HIV-infected women with variable transfer efficiency**

455     Finally, to examine the combined impact of IgG characteristics on transplacental transfer

456 efficiency, we fit a linear regression model that combined each of the identified characteristics of

457 gp120, tetanus toxoid, and pertussis toxin-specific IgG found to be associated with placental IgG

458 transfer efficiency in U.S. HIV-infected women with variable transplacental IgG transfer

459 efficiency. We related these IgG characteristics to their unique antigen-specific transplacental

460 IgG transfer score by multivariable linear regression. Again, due to the high collinearity of IgG1

461 subclass responses and antigen-specific IgG response magnitude, the frequency of IgG1 subclass

462 was not included in the combined model of IgG characteristics (Table 7). The frequency of

463 tetanus toxoid-specific IgG subclass IgG4 responses was also negatively associated with

464 transplacental IgG transfer of tetanus toxoid-specific IgG (slope: -0.65, $p < 0.01$) (Table 7). The

465 high frequency of IgG4 subclass responses and the negative association with transplacental IgG

466 transfer was only observed for tetanus toxoid-specific IgG. Moreover, in the combined model of

467 IgG Fc characteristics, the frequency of IgG2 subclass responses was strongly positively

468 associated with transplacental IgG transfer efficiency of gp120, tetanus toxoid, and pertussis

469 toxin-specific IgG (slope: 1.21, $p < 0.01$, slope: 0.99, $p < 0.01$, slope: 1.26, $p < 0.01$, respectively). In

470 addition, magnitude of maternal plasma gp120-specific IgG was positively associated with

471 transplacental IgG transfer efficiency this specificity (slope: 0.27, $p < 0.03$). Finally, gp120-

472 specific IgG Fc region glycan profiles that comprised PC2 remained weakly positively associated

473 with transplacental IgG transfer (slope: 0.26, $p < 0.01$). Altogether, these findings suggest that IgG

474 Fc characteristics differentially mediate the selective placental transfer efficiency of distinct IgG

475 subpopulations.

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476

## DISCUSSION

477

478    A major roadblock for improving the protection of newborns via maternal passively-

479    acquired maternal IgG is our limited understanding of the molecular interactions of IgG and

480    placental FcRs that modulate transplacental IgG transfer. Placental IgG transfer is impaired in

481    the setting of certain maternal infections, such as HIV and malaria (Cumberland et al., 2007; de

482    Moraes-Pinto et al., 1996; de Moraes-Pinto et al., 1993; de Moraes-Pinto et al., 1998b; Palmeira

483    et al., 2012). Thus, we sought to define factors that associated with poor or efficient placental

484    IgG transfer in the pathologic setting of maternal HIV-infection. Interestingly, we observed wide

485    variability in the efficiency of transplacental transfer of antigen-specific IgG in U.S. and

486    Malawian HIV-infected women who received minimal ART treatment (Figure 1). In U.S. HIV-

487    infected mother infant pairs, we observed efficient, poor, and variable transplacental IgG

488    transfer. In contrast, in Malawian HIV-infected mother infant pairs, we only observed poor and

489    variable transplacental IgG transfer. It was surprising to observe the variable transplacental IgG

490    transfer efficiency phenotype in the majority of HIV-infected women, and it suggests that the

491    placental transfer is distinctly regulated for different antigen-specific IgG populations. Malawian

492    HIV-infected women had more evidence of advanced HIV disease, with higher plasma viral

493    load, lower peripheral blood CD4 + T cell counts, and higher concentrations of total plasma IgG

494    compared to HIV-infected women from the U.S. (Table 1), potentially accounting for the lack of

495    an efficient transplacental IgG transfer efficiency in this cohort.

496    Previous studies have reported the suboptimal transplacental transfer of protective IgG in

497    HIV-infected women (Abu-Raya et al., 2016; Gupta et al., 2014; Jones et al., 2011), raising the

498    possibility that the poor transplacental transfer of maternal protective IgG may contribute to the

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499   higher rates of infectious diseases in HEU infants. Accordingly, we observed that between 7-

500   79% of infants born to HIV-infected women with protective plasma IgG titers against tetanus,

501   rubella, diphtheria, and Hib, were born with IgG concentrations below protective thresholds

502   against these common neonatal pathogens (Figure 2). Interestingly, despite having a tetanus

503   toxoid booster vaccine during pregnancy, Malawian HIV-infected women still had overall poor

504   transplacental IgG transfer of tetanus toxoid-specific IgG. Moreover, up to 15% of HEU infants

505   born to Malawian HIV-infected women were below the protective titer for tetanus-specific IgG

506   at birth, suggesting that maternal vaccination alone may not be sufficient to overcome the poor

507   transplacental IgG transfer in this population. These findings highlight that the poor

508   transplacental transfer of maternal protective IgG in HIV-infected pregnant women puts their

509   HEU infants at risk for vaccine-preventable infectious diseases.

510       In our study, biomarkers of maternal HIV disease progression, including CD4+ T cell

511   counts and hypergammaglobulinemia, were associated with poor placental IgG transfer.

512   However, maternal plasma HIV load was not associated with transplacental IgG transfer

513   efficiency in either U.S. or Malawian HIV-infected women. This finding contrasts with a prior

514   study that reported maternal plasma HIV load and poor transplacental IgG transfer efficiency in a

515   cohort of 50 HIV, clade A virus-infected Kenyan women (Farquhar et al., 2005). This disparate

516   finding could be due to differences in maternal HIV disease progression and potentially HIV

517   clade-specific differences. Total IgG concentrations in maternal plasma above a certain high

518   threshold (greater than ~15mg/ml) have previously been associated with poor transplacental IgG

519   transfer efficiency (Gonçalves et al., 1999; Hartter et al., 2000; Michaux et al., 1966; Okoko et

520   al., 2001; Palmeira et al., 2012), and accordingly, maternal hypergammaglobulinemia was

521   associated with poor transplacental IgG transfer efficiency in both U.S. and Malawian HIV-

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522    infected women (Table 3). In addition, Malawian HIV-infected women had higher median

523    concentrations of plasma total IgG concentrations compared to U.S. HIV-infected women (Table

524    1). Altogether, our findings suggest that low maternal peripheral blood CD4+ T cell counts and

525    hypergammaglobulinemia negatively impact the transplacental transfer of maternal IgG. While it

526    is possible that high maternal plasma IgG concentrations are a biomarker of advanced HIV-

527    disease progression that tracks together with low peripheral blood CD4 + T cell counts in these

528    primarily untreated cohorts, it is also possible that maternal high plasma IgG concentrations lead

529    to impaired transplacental IgG transfer by saturating placentally expressed FcRs that shuttle

530    maternal IgG to the fetus (Englund, 2007; Palmeira et al., 2012; Wilcox et al., 2017). In addition,

531    it is possible that the B cell dysfunction that leads to hypergammaglobulinemia in HIV-infected

532    women also results in altered Fc region characteristics (i.e., altered IgG subclass composition or

533    Fc region glycan profiles) that lead to impaired transplacental transfer of some IgG

534    subpopulations.

535        While the role of placentally expressed FcRn in mediating the transplacental transfer of

536    maternal IgG is clear (Palmeira et al., 2012; Roopenian and Akilesh, 2007; Simister and Mostov,

537    1989), the role of noncanonical placental FcRs in transplacental transfer of maternal IgG remains

538    unexplored (Simister, 2003; Simister and Story, 1997). We did not detect overall placental FcR

539    expression differences in HIV-infected, Malawian women with distinct phenotypes of

540    transplacental IgG transfer (Figure S2, Figure S3), suggesting the determinants of IgG transfer

541    efficiency are more likely to be modulated by IgG characteristics or other factors, even though it

542    remains unclear if cell-type-specific FcR abundances may play a role. Moreover, differences in

543    the overall placental FcRs expression levels in Malawian HIV-infected women were not

544    associated with transplacental IgG transfer efficiency. Interestingly, the binding of tetanus

23

545    toxoid-specific IgG to placentally expressed FcγRIIa 131H, FcγRIIa 131R, and FcγRIIIa F158,

546    positively associated with transplacental IgG transfer efficiency in Malawian HIV-infected

547    women, suggesting that IgG interaction with noncanonical placental FcRs may play a role in

548    mediating the transplacental transfer of some antigen-specific IgG populations (Table 4). The

549    distinct outcomes in FcR binding and transplacental transfer efficiency among maternal gp120,

550    tetanus toxoid, and pertussis toxin-specific IgG, may be explained by differences in IgG

551    characteristics among these distinct antibody populations. For example, tetanus toxoid-specific

552    IgG responses in Malawian HIV-infected women had higher frequencies of IgG4 subclass

553    responses compared to both gp120 and pertussis-specific IgG responses (Figure S6).

554    Furthermore, gp120, tetanus toxoid, and pertussis-specific IgG exhibited distinct Fc region

555    glycan profiles (Figure 3) which could contribute to the lack of a uniform binding strength to

556    distinct placentally-expressed Fc receptors (Figure S2).

557        Maternal IgG subclass is known to be an important factor in transplacental IgG transfer

558    (Fouda et al., 2018; Palmeira et al., 2012), with IgG1 and IgG4 being the most efficiently

559    transferred (Garty et al., 1994). In this cohort of U.S. HIV-infected women, the frequency of

560    gp120-specific IgG3 subclass, tetanus toxoid-specific IgG1, and pertussis-specific IgG4 subclass

561    responses negatively associated with transplacental transfer efficiency (Table 5), suggesting that

562    IgG subclass frequency of some antigen-specific IgG populations may negatively impact the

563    transplacental IgG transfer. Moreover, maternal IgG2 subclass frequency of gp120, tetanus

564    toxoid, and pertussis toxin-specific IgG strongly positively associated with efficient

565    transplacental IgG transfer in the combined model (Table 7) of IgG characteristics (Table 5).

566    However, the association of gp120, tetanus toxoid, and pertussis toxin-specific IgG subclass

567    prevalence and efficient transplacental IgG transfer should be interpreted with caution given the

24

568    low frequency of IgG2 subclass responses in U.S. HIV-infected women with variable

569    transplacental IgG transfer efficiency. We did not find the same association of IgG subclass

570    frequencies and transplacental IgG transfer in Malawian HIV-infected women, potentially

571    attributable to a smaller cohort size or more advanced HIV disease progression. It is also possible

572    that higher frequency of IgG1 and IgG4 subclass responses track together with

573    hypergammaglobulinemia in HIV-infected women. As it has been reported that IgG subclasses

574    have distinct affinity for FcRn, FcγRII, and FcγRIII (Abdiche et al., 2015; Bruhns et al., 2009),

575    IgG subclass-specific affinity to placental FcRs could be an important, yet unexplored,

576    determinant of transplacental IgG transfer efficiency.

577         The Fc region glycosylation profile at the conserved glycosylation site may also be an

578    important factor that impacts transplacental IgG transfer efficiency, due to its role in mediating

579    IgG binding to certain placental Fc receptors including: FcγRI, FcγRII, and FcγRIII. While the

580    affinity of IgG Fc to the canonical IgG placental shuttle receptor, FcRn, is not likely dependent

581    on this Fc glycosylation profile (Martin et al., 2001), previous studies have reported differences

582    in IgG Fc region glycans in mother infant pairs (Williams et al., 1995), suggesting that IgG Fc

583    glycan-dependent placentally-expressed Fcγ receptors may modulate transplacental IgG transfer

584    efficiency. While one study that examined IgG Fc region glycans in ten mother-infant pairs

585    found no significant differences in Fc region glycosylation patterns in maternal and infant IgG

586    populations (Einarsdottir et al., 2013), a more recent study found overall IgG glycan differences

587    in maternal IgG and those that were passively transferred to the infant (Jansen et al., 2016).

588    However, these studies examined glycosylation profiles in total maternal IgG as opposed to that

589    of antigen-specific IgG populations, potentially masking distinctions in placentally transferred Fc

590    region glycan profiles. Consistent with a previous study that reported distinct Fc region glycans

591    profiles of antigen-specific IgG populations (Mahan et al., 2016), poorly transplacentally-

592    transferred gp120-specific IgG had overall differences in the relative amounts of certain Fc

593    region glycans compared to that of efficiently-transferred tetanus and pertussis toxoid-specific

594    IgG (Figure 4). Further, we identified that the Fc region glycan profile was associated with

595    transplacental IgG transfer efficiency of gp120-specific IgG (Figure 4, Table 6). Fucosylated,

596    bisected, and disialylated IgG Fc region glycans were weakly associated with efficient

597    transplacental IgG transfer efficiency of gp120-specific IgG in combination, but had limited

598    contribution individually (Table 6), suggesting a cooperative effect among Fc region glycans. Fc

599    region fucose glycans have been shown to mediate binding strength to FcγRIIIa in vitro (Okazaki

600    et al., 2004). Accordingly, we also found that poorly transferred gp120-specific IgG had lower

601    frequencies of Fc region fucose, suggesting that Fc region glycans modulate transplacental IgG

602    transfer efficiency, likely through altered binding to placental Fc receptors such as FcγRIIIa. Our

603    findings suggest that antigen-specific IgG Fc profiles modulate the selective placental transfer

604    efficiency of distinct IgG subpopulations.

605        While we aimed to comprehensively examine factors that mediate the transplacental IgG

606    transfer in HIV-infected women, our study has some limitations. Both cohorts lacked detailed

607    vaccination history, with the exception of tetanus vaccination during pregnancy in Malawian

608    women. Another limitation of our study is the lack of a robust clinical record for U.S. HIV-

609    infected women of potential co-morbidities that could also impact the efficiency of transplacental

610    IgG transfer. Nonetheless, we examined the role of clinical signs of preeclampsia and maternal

611    syphilis in Malawian HIV-infected women and designed our linear regression models to correct

612    for these factors that are known to affect transplacental IgG transfer. Moreover, while we found

613    that placental FcR RNA expression levels were not distinct among HIV-infected Malawian

614     women with variable and poor transplacental IgG transfer, it is known that gene copy numbers

615     may vary within the FcR locus and could complicate the interpretation of FcR mRNA expression

616     levels (Hollox and Hoh, 2014; Lassauniere and Tiemessen, 2016). Our inability to detect FcR

617     RNA expression level differences could also be due to the absence of an efficient transplacental

618     IgG transfer group in HIV-infected Malawian women. Another caveat to our study is that factors

619     in U.S. HIV-infected women that were associated with transplacental IgG transfer efficiency

620     were not always similarly correlated with transplacental IgG transfer efficiency in Malawian

621     HIV-infected women. While these differences could be explained by differences in cohort sizes,

622     health, and/or genetic differences in U.S. and Malawian HIV-infected women, our findings

623     should be validated in larger cohorts of HIV-infected mother infant pairs. It should also be noted

624     that the calculated transplacental IgG transfer score was specifically designed to detect

625     differences among HIV-infected U.S. and Malawian women with distinct IgG transfer

626     phenotypes and was inherently arbitrary. Finally, as only 5 of 167 infants were HIV infected, our

627     study was not designed to identify immune correlates of protection against mother to child

628     transmission of HIV, but instead was geared towards defining determinants of transplacental IgG

629     transfer efficiency.

630         Altogether, our findings reveal that the placental transfer of maternal IgG is a selective

631     process, particularly in the setting of HIV infection. Our results suggest that a combination of

632     factors, including IgG FcR binding strength, subclass, and glycan profiles including fucose, but

633     not placental FcR expression levels or antigen-specific IgG concentration, collectively play a

634     role in the selective and differential placental transfer of maternal antigen-specific IgG (Figure

635     5). Our results also suggest that the transplacental transfer efficiency is differentially regulated

636     by distinct Fc region characteristics across antigen-specific IgG subpopulations. These findings

637    also shed light on the potential design of strategies to improve the transplacental IgG transfer to

638    the fetus via Fc region modifications of some antigen-specific IgG (i.e., tetanus toxoid and

639    pertussis toxin-specific IgG). Moreover, our findings have important implications for improving

640    the transplacental IgG transfer achieved by routinely administered vaccine-elicited IgG responses

641    in pregnancy such as the tetanus, diphtheria, and pertussis vaccine (i.e., Tdap), especially in

642    HIV-infected women. For example, future studies could explore adjuvant-mediated modulation

643    of the Fc region characteristics of vaccine-elicited IgG to increase binding to placentally-

644    expressed FcRs or drive specific Fc region glycosylation profiles with the goal of improving

645    transplacental IgG transfer efficiency. These data also suggest that maternal HIV treatment that

646    prevents CD4+ T cell loss and reduces maternal hypergammaglobulinemia is likely to improve

647    the transplacental transfer of maternal protective IgG, which should be studied in combination

648    ART-treated HIV-infected maternal populations. Future work that further defines the

649    determinants of placental IgG transport will ultimately inform strategies to improve the transfer

650    of maternal IgG to the vulnerable fetus, extending the window of maternal IgG-mediated infant

651    protection in the first year of life.

652

653    **EXPERIMENTAL PROCEDURES**

654    **Study population**

655    HIV-infected women and their HIV-infected or HIV-exposed uninfected (HEU) infants were

656    selected from two previously described maternal-infant cohorts based on the availability of

657    maternal plasma and paired infant cord blood plasma sample from delivery (Fouda et al., 2013;

658    Permar et al., 2015; Sacha et al., 2015). N=120 maternal and infant cord-blood samples from

659    delivery were obtained from the Woman and Infant Transmission Study (WITS) cohort. WITS

660    study participants were HIV-infected women from North America, thus primarily infected with

661    clade B virus. All 120 infants included from the WITS cohort were HEU as determined by

662    plasma HIV load PCR testing at delivery. Forty-seven maternal and infant cord blood plasma

663    samples from delivery were obtained from the Center for HIV/AIDS Vaccine Immunology 009

664    (CHAVI009) cohort of HIV-infected women from Malawi. Mothers and infants enrolled in the

665    CHAVI009 study received a single dose Nevirapine at delivery. Five infants from the

666    CHAVI009 cohort were HIV-infected *in utero,* and n=42 infants were HEU as determined by

667    whole blood HIV DNA PCR testing at delivery.

668    **Ethics statement**

669    Approval was obtained from the institutional review board at each collaborating institution and

670    enrollment clinical sites to utilize de-identified maternal and infant cord blood plasma samples

671    from the WITS and CHAVI009 cohorts.

672    **Measurement of antigen-specific IgG levels**

673    A binding antibody multiplex assay (BAMA) was used to measure maternal and infant HIV and

674    non-HIV-specific IgG responses. Briefly, carboxylated beads were coupled to HIV and non-HIV

675    antigens as described previously (Tomaras et al., 2008). A total of $5 \times 10^6$ carboxylated

676    fluorescent beads (Luminex Corp) were covalently coupled to 25µg of proteins and peptides

677    tested. Recombinant HIV and non-HIV antigens included: Con6gp120, MNnegD11gp120,

678    gp70MNV3, gp70BcaseAV1V2, MulVgp70_his, Bio-V3.C (Bio-

679    KKKNNTRKSIRIGPGQTFYATGDIIGDIRQAHC), Bio-MPER03 (Bio-

680    KKKNEQELLELDKWASLWNWFDITNWLWYIR), Bio-MPER656, (Bio-

681    KKKNEQELLELDKWASLWNWFNITNWLW), Tetanus toxoid (Pfenex, Inc), Pertussis toxin

682    (Sigma-Aldrich), Influenza hemagglutinin/A/Solomon Islands/03/2006 (Protein Sciences Corp),

683    Rubella virus capsid (Abcam), Diphtheria toxin (Sigma), *Hemaphilus Influenza B* (Hib)Type B

684    oligosaccharide-Human Serum Albumin conjugate (BEI Resources), Hepatitis B surface antigen

685    (Abcam), and Respiratory syncytial virus (RSV) F surface antigen (DSCAV-1) (a kind gift of Dr.

686    Barney Graham, NIH VRC). Peptides were purchased from CPC Scientific (Sunnyvale, CA).

687    Maternal and infant plasma was tested against Con6gp120, MNnegD11gp120, gp70MNV3,

688    gp70BcaseAV1V2, MulVgp70_his, Bio-V3.C, Bio-MPER03, Bio-MPER656, Tetanus toxoid,

689    Pertussis toxin, Influenza hemagglutinin/A/Solomon Islands/03/2006, Rubella virus capsid,

690    Diphtheria toxin, and RSV DSCAV-1 surface F antigen at 1:100 dilution in serum diluent (1X

691    PBS, 1% milk, 5% normal goat serum, 0.05% Tween-20). Maternal and infant plasma was tested

692    against Hib Type B oligosaccharide-Human Serum Albumin conjugate and Hepatitis B surface

693    antigen at a 1:25 dilution in a modified serum diluent to reduce background (1X PBS, 1% milk,

694    5% normal goat serum, 0.05% Tween-20, 0.05% polyvinyl alcohol, and 0.08%

695    polyvinylpyrrolidone). Maternal and infant plasma HIV and vaccine antigen-specific IgG was

696    detected with a mouse anti-human IgG (Southern Biotech, Birmingham, AL) phycoerythrin-

697    conjugated antibody at 2µg/ml as described previously (Tomaras et al., 2008).

698    Hyperimmuneglobulin isolated from HIV-seropositive donors (HIVIG) standard was used to

699    calculate concentration of Con6gp120-specific IgG antibodies in mother infant pairs. A

700    membrane proximal external region (MPER)-specific IgG (2F5) was used to calculate the

701    concentration of MPER-specific IgG in mother infant pairs. A V3-specific IgG (CH22) was used

702    to calculate the concentration of maternal and infant V3-specific IgG. A V1V2-specific IgG

703    (CH58) was used to calculate the concentration of V1V2-specific IgG in mother infant pairs. An

704    influenza hemagglutinin-specific IgG (CH65) was used to calculate the concentration of flu-

705    specific IgG in mother infant pairs. Finally, an RSV surface antigen-specific mAb (Palivizumab -

30

706 MedImmune) was used to calculate the concentration of RSV-specific IgG in mother infant

707 pairs. Concentration of tetanus toxoid, pertussis toxin, rubella virus capsid, *Haemophilus*

708 *influenzae* type B, hepatitis B, and diphtheria toxin-specific IgG was determined by using

709 commercially available WHO international standards (National Institute for Biological Standards

710 and Controls – anti-tetanus toxoid sera: 26/288, anti-pertussis sera: 06/140, anti-rubella sera:

711 91/688, anti-Haemophilus influezae type B: 09/222, anti-Hepatitis B: 07/164, anti-diphtheria

712 toxin: 10/262). We measured protective concentration thresholds in U.S. and Malawian HIV-

713 infected women as set by the World Health Organization (WHO) (Plotkin, 2010): tetanus toxoid-

714 specific IgG (0.10 IU/ml), rubella-specific IgG (10 IU/ml), diphtheria toxin-specific IgG (0.10

715 IU/ml), and Hib-specific IgG (0.15 μg/ml). Normal human sera samples from uninfected donors

716 were used as a negative control for HIV antigens. All MFI values were blank bead and well

717 subtracted. All mother-infant samples were tested at the same dilution. Antibody measurements

718 were acquired using a Bio-Plex 200 instrument (Bio-Rad).

719 **Calculation of transplacental IgG transfer efficiency**

720 Maternal IgG transplacental transfer efficiency was calculated as infant antigen-specific IgG

721 concentration/maternal antigen-specific IgG concentration X 100. Efficient transplacental

722 transfer was defined as >99% transfer efficiency for the majority (>75%) of measured antigen-

723 specific IgG. Variable transplacental transfer was defined as efficient (>99%) and poor (<60%)

724 transfer efficiency of measured antigen-specific IgG within a mother infant pair. Poor

725 transplacental transfer was defined as <60% transfer efficiency for the majority (>75%) of

726 measured antigen-specific IgG.

727 **Measurement of HIV and non-HIV antigen-specific IgG subclass**

728     To measure HIV and vaccine-antigen IgG subclass responses, maternal samples were tested

729     against a Con6gp120, MNgDneg11gp120, gp70MNV3, gp70BcaseAV1V2, tetanus toxoid,

730     pertussis toxin, MulVgp70_his, influenza hemagglutinin/A/Solomon Islands/03/2006 (Protein

731     Sciences Corp), and a blank bead at a 1:40 dilution by BAMA. Biotinylated antibody reagents

732     specific for each IgG subclass were used at 4 µg/ml: anti-IgG1-biotin (BD Pharmingen, clone:

733     G17-1), anti-IgG2-biotin (BD Pharmingen, G18-21), anti-IgG3-biotin (Calbiochem, clone:

734     HP6047), and anti-IgG4-biotin (BD Pharmingen, clone: G17-4). Plates were washed 3X and

735     streptavidin-conjugated phycoerythrin antibody diluted was used at 1:100 dilution and added to

736     wells. All MFI values were blank subtracted.

737     **IgG Fc region glycosylation analysis**

738     Maternal plasma samples were heat inactivated at 56°C for 1 hour. Maternal plasma samples

739     were incubated with 25µl of uncoated streptavidin coated magnetic beads (New England

740     Biolabs) at room temperature (RT) in rotation. MNnegD11gp120, tetanus toxoid, and pertussis

741     toxin antigens were biotinylated with LC-LC-biotin (Thermo Scientific) according to the

742     manufacturer's protocol. Streptavidin coated magnetic beads were activated with 0.5M NaCl,

743     20mM Tris-HCl [pH 7.5], 1mM EDTA and incubated with biotinylated antigens at RT for 1 hour

744     in rotation. Maternal plasma and coupled beads were mixed and incubated at RT for 1 hour in

745     rotation. Adsorbed plasma samples were removed and antigen-specific IgG bound to the antigen-

746     coupled beads was digested with IDEZ (New England Biolabs) at 37°C for 1 hour in rotation.

747     Cleaved Fc region supernatants were digested with PNGaseF (New England Biolabs) according

748     to manufacturer specifications. To precipitate Fc region glycans, ice-cold ethanol was added and

749     incubated at -20°C for 30 min. Plates were spun and the ethanol layer was transferred to clean

750     plates. Plates were spun in a Centrivap (Labconco Corporation) and dried glycans were labeled

32

751    with APTS dye (Life Technology) in Sodium Cyanoborohydride in Tetrahydrofuran (Sigma).

752    Labeled glycans were transferred to filtered plates (Harvard Apparatus) containing P-2 slurry

753    (Bio-Rad) and excess APTS dye was removed by washing with water. Cleaned Fc region labeled

754    glycans were run immediately on a 3130 Genetic analyzer Sanger sequencer (Applied

755    Biosystems) as described previously (Mahan et al., 2015).

756    **Measurement of total plasma IgG**

757    Goat anti-human polyclonal IgG (Life Technologies) was used to coat high binding 384 well

758    ELISA plates (Corning) with 3µg/ml overnight at 4°C. Plates were washed and blocked with

759    SuperBlock (4% whey protein, 15% goat serum, and 0.5% Tween 20 diluted in 1X PBS) for 2

760    hours at RT. Maternal plasma IgG was tested at 1:1000 starting dilution and was serially diluted

761    in 3-fold dilutions. Reagent grade normal human serum IgG (Sigma) was used as a standard at a

762    starting concentration of 10µg/ml and was serially diluted in 3-fold dilutions. An anti-human IgG

763    HRP conjugated antibody produced in goat (Sigma Aldrich) was used at 1:10,000 dilution. After

764    washing the plates, SureBlue reserve TMB substrate (KPL) was added. Substrate reactions were

765    stopped by adding an equal volume of Stop solution (KLP). Optical densities were read at 450nm

766    using a Spectramax plate reader. OD values within the linear range of a 5-parameter standard

767    curve were used to interpolate the total IgG concentration of plasma samples.

768    **Placental RNAseq analysis and assessment of FcR expression levels**

769    Placental biopsy samples in RNAlater from HIV-infected Malawian women were thawed on ice.

770    Fifty mg of tissue was homogenized in Trizol (Invitrogen), and total RNA was purified with

771    miRNeasy extraction kit (Qiagen) according to the manufacturer guidelines. RNA quality was

772    assessed on Bioanalyzer 2100 instrument using RNA 6000 Nano Kit (Agilent). Total RNA

773    libraries were prepared from 500 ng purified RNA using TruSeq Stranded Total RNA with Ribo-

33

774     Zero Gold Kit (Illumina). Deep sequencing was performed on a Nextseq500 sequencer

775     (Illumina) using 75bp paired-end reads. Raw BCL (base call) files generated from NextSeq

776     sequencer were converted to FASTQ files using bcl2fastq Conversion Software v2.18. During

777     BCL to FASTQ processing, bcl2fastq also separates multiplexed samples and removes adapters.

778     Each pair of a FASTQ file was sequentially mapped to human ribosomal RNA and hemoglobin

779     sequences using a gapped aligner STAR (Dobin et al., 2013) to make sure that rRNA was

780     depleted and there was no hemoglobin contamination. Raw RNA-seq data in FASTQ file format

781     was quality controlled during and after sequencing to identify potential technical issues. Cleaned

782     sequencing reads of placental FcRs were then mapped to the human reference genome (assembly

783     GRCh38, Gencode annotation release 25) using STAR to generate read counts for each of

784     annotated genes. The raw gene read count data was normalized using edgeR (Robinson et al.,

785     2010).

786     **FcR binding antibody multiplex assay**

787     Fv and Fc profiles of plasma antibodies were characterized using a multiplexed Fc array assay as

788     described previously (Brown et al., 2017; Brown et al., 2018). In brief, recombinant proteins

789     were covalently coupled to fluorescent beads (Luminex). To display biotinylated peptides,

790     streptavidin (Rockland) was coupled to beads first to capture the peptides. In duplicate, test

791     samples were diluted 1:250 or 1:1,000 into a 384-well microplate (Greiner Bio One) containing

792     ~500 beads of each specificity per well. Following bead opsonization, beads were washed and

793     subsequently incubated with PE-conjugated Fc detection reagents, including human FcRs

794     tetramers (Boesch et al., 2014), anti IgG (Southern Biotech), and C1q (Sigma-Aldrich), followed

795     by a final wash. Data were acquired on a FlexMap3D instrument (Luminex) and raw data were

796     reported as MFI values.

797 **Statistical analysis**

798 The statistical analysis plan was finalized prior to data analysis. Multivariable linear regression

799 models with sandwich variance estimates were used to evaluate the association between transfer

800 efficiency and covariates of interest. Before regression, transfer efficiency was rank-gauss

801 transformed (i.e. they are ranked and the ranks are transformed by the inverse Gaussian

802 probability function). Missing antibody-specific transfer efficiencies were imputed using the R

803 package mice. Principal component analysis of glycan markers was performed after the markers

804 were scaled to 1. Global transplacental transfer was calculated for each patient and assigned a

805 transfer score. An antigen-specific IgG transplacental transfer score was assigned to each distinct

806 IgG specificity. All statistical procedures were implemented using the R language and

807 environment for statistical computing and graphics. False discovery rate (FDR) p values are

808 reported as FDR values.

809

835

836    **Author Contributions:**

837    D.R.M., G.G.F., and S.R.P. conceptualized and conceived the study. D.R.M., S.H.L., F.Y.,

838    J.A.W., E.A.H., R.G., J.F.M., and M.J. performed experiments and analyzed the data with help

839    from Y.F., G.A., M.E.A., X.P., G.G.F., S.R.P.. G.S. provided critical samples for the study. All

840    authors discussed the results and commented on the manuscript. Funding acquisition: D.R.M.,

841    X.P., G.G.F., and S.R.P.

842    **Declaration of interests.** S.R.P., is a consultant for Pfizer, Moderna, and Merck vaccine

843    programs. All other authors declare no competing interests.

844

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1099    **Tables**

**Table 1. Clinical characteristics of U.S. and Malawian HIV-infected women and their infants**

| Geographic location | Cohort | Mother infant pairs N | Maternal ART treatment during pregnancy N, (%) | Maternal ART treatment at delivery N, (%) | Maternal peripheral blood CD4+ T cell count (cells/mm$^3$) | Maternal plasma viral load (copies/ml) | Maternal total serum IgG (mg/ml) | HIV-exposed uninfected infants N, (%) | HIV-infected infants N, (%) | Infant gestational age (weeks) | Infant weight (Kg) | Infant sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| U.S. | WITS | 120 | 10, (8.3%) | 2, (1.6%) | 642 (32-2,330) [a] | 33,929 (50-905,230) | 16.3 (2.5-123.5) | 120, (100%) | 0, (0%) | 40 (30-43) | 3.1 (1.4-4.5) | 41% F |
| Malawi | CHAVI009 | 47 | 13, (28%) | 47, (100%) | 381 (94-833) | 42,272 (400-343,721) | 39.9 (8.5-158.4) | 42, (89.3%) | 5, (10.6%) | 39 (33-44) | 3.1 (2.3-4.04) | 55% F |

[a] Median and range are shown

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**Table 2. Associations between maternal plasma HIV and vaccine antigen-specific IgG levels and transplacental IgG transfer efficiency in HIV-infected pregnant women**

| Cohort | HIV gp120 | HIV MPER | HIV V3 | HIV V1V2 | Influenza HA | Pertussis toxin | Tetanus toxin | Diphtheria toxin | Rubella virus capsid | Hep B | RSV | Hib |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| U.S. | 0.08 (0.47) [a] | -0.07 (0.51) | **-0.24 (0.05)** | -0.06 (0.57) | -0.11 (0.26) | 0.08 (0.45) | -0.04 (0.67) | 0.16 (0.11) | 0.06 (0.57) | 0.02 (0.78) | **-0.32 (0.001)** | 0.21 (0.070) |
| Malawian | 0.00 (0.98) | -0.22 (0.29) | -0.13 (0.50) | -0.24 (0.11) | -0.07 (0.73) | 0.19 (0.18) | **0.50 (0.004)** | -0.10 (0.52) | -0.05 (0.74) | 0.05 (0.72) | 0.00 (0.99) | 0.12 (0.62) |

[a] Estimated regression slopes with p values in parentheses

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## Table 3. Associations between maternal HIV disease progression and infant clinical characteristics and transplacental IgG transfer efficiency

| Maternal clinical factor/Infant characteristic at birth | U.S. | | | Malawian | | |
|---|---|---|---|---|---|---|
| | slope[a] | raw p | FDR[b] | slope | raw p | FDR |
| Maternal ART treatment during pregnancy | 0.01 | 0.96 | 0.96 | -0.03 | 0.53 | 0.85 |
| **Maternal peripheral blood CD4+ T cell count** | **0.19** | **0.002** | **0.004** | -0.03 | 0.85 | 0.85 |
| Maternal plasma HIV RNA load | 0.02 | 0.74 | 0.93 | -0.05 | 0.8 | 0.85 |
| **Maternal total plasma IgG level (hypergammaglobulinemia)** | **-0.37** | **0.001** | **0.001** | **-0.4** | **0.005** | **0.02** |
| Infant gestational age | 0.04 | 0.62 | 0.62 | 0.24 | 0.03 | 0.1 |
| Infant weight | 0.1 | 0.19 | 0.55 | -0.33 | 0.05 | 0.1 |
| Infant sex | -0.08 | 0.57 | 0.62 | 0.08 | 0.74 | 0.74 |

[a] Slope: Estimated regression slope values

[b] FDR: false discovery rate adjusted p value

Bolded values indicate statistical significance

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## Table 4. Associations of transplacental IgG transfer efficiency and maternal plasma antigen-specific IgG binding to placentally-expressed Fc receptors in Malawian women

| Factor | Malawian | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | gp120 | | tetanus toxoid | | pertussis toxin | |
| | slope | p value | slope | p value | slope | p value |
| All Fc receptors* | 0 | 0.75 | 0.23 | 0.05 | 0.02 | 0.95 |
| FcRn | | | 0.26 | 0.12 | | |
| FcγRIIa H131 | | | 0.18 | 0.03 | | |
| FcγRIIa R131 | | | 0.15 | 0.03 | | |
| FcγRIIb | | | 0.15 | 0.08 | | |
| FcγRIIIa V158 | | | 0.36 | 0.12 | | |
| FcγRIIIa F158 | | | 0.21 | 0.05 | | |
| FcγRIIIb | | | 0.12 | 0.1 | | |

Not calculated because of no statistical significance in the combined analysis against all Fc receptors* (row 1) (raw p>0.05)
Bolded values indicate statistical significance

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## Table 5. Associations of transplacental IgG transfer efficiency and antigen-specific IgG subclass responses in U.S and Malawian HIV-infected women with variable transplacental IgG transfer

| Cohort | Maternal IgG subclass | U.S. | | | | | |
| | | gp120-specific IgG transfer | | tetanus toxoid-specific IgG transfer | | pertussis toxin-specific IgG transfer | |
| | | slope | p value | slope | p value | slope | p value |
| --- | --- | --- | --- | --- | --- | --- | --- |
| U.S. | Antigen-specific IgG frequency [b] | 0.03 | 0.82 | -0.09 | 0.41 | **-0.33** | **0.02** |
| | IgG2 frequency | 1.78 | 0.08 | 0.78 | 0.15 | 0.70 | 0.44 |
| | IgG3 frequency | **-0.68** | **0.03** | 0.09 | 0.81 | -0.27 | 0.61 |
| | IgG4 frequency | LF [a] | LF | **-0.52** | **0.04** | 0.15 | 0.87 |
| Malawian | Antigen-specific IgG frequency | -0.79 | 0.28 | 0.35 | 0.11 | LF | LF |
| | IgG2 frequency | -0.45 | 0.63 | 0.09 | 0.84 | 0.83 | 0.39 |
| | IgG3 frequency | 0.28 | 0.74 | 0.18 | 0.64 | 0.52 | 0.22 |
| | IgG4 frequency | 0.12 | 0.87 | -0.02 | 0.97 | -0.25 | 0.80 |

[a] LF: low frequency.

[b] Due to the high collinearity of IgG1 subclass responses and antigen-specific IgG response magnitude, the frequency of IgG1 subclass was not included in the combined model of IgG characteristics

Bolded values indicate statistical significance

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**Table 6. Associations transplacental IgG transfer efficiency and antigen-specific plasma IgG levels and Fc region glycan profiles in U.S. women with variable transplacental IgG transfer**

| Maternal IgG characteristic | U.S. | | | | | |
|---|---|---|---|---|---|---|
| | gp120 | | tetanus toxoid | | pertussis toxin | |
| | slope | p value | slope | p value | slope | p value |
| Fc region glycans PC1 | 0.11 | 0.31 | 0.13 | 0.20 | -0.22 | 0.09 |
| Fc region glycans PC2 | **0.27** | **0.01** | -0.07 | 0.68 | 0.04 | 0.78 |
| G0 | 0.01 | 0.46 | | | | |
| G1 | -0.03 | 0.13 | | | | |
| G2 | 0.00 | 0.72 | | | | |
| Fucose | **-0.02** | **0.03** | | | | |
| Bisecting | 0.02 | 0.39 | | | | |
| Di-sialic acid | **0.07** | **0.003** | | | | |
| Mono-sialic acid | 0.01 | 0.50 | | | | |
| Total-sialic acid | 0.01 | 0.12 | | | | |

■ Not calculated because of no statistical significance of association with Fc glycan PC1 and PC2
Bolded values indicate statistical significance

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## Table 7. Combined analysis of maternal IgG characteristics associated with transplacental IgG transfer efficiency in U.S HIV-infected women with variable transplacental IgG transfer efficiency

| Maternal IgG characteristic | U.S. | | | | | |
|---|---|---|---|---|---|---|
| | gp120 | | tetanus toxoid | | pertussis toxin | |
| | slope | p value | slope | p value | slope | p value |
| IgG2 frequency | **1.21** | **0.01** | **0.99** | **0.01** | **1.26** | **0.01** |
| IgG3 frequency | -0.45 | 0.21 | 0.10 | 0.71 | -0.52 | 0.23 |
| IgG4 frequency | LF [a] | LF | **-0.65** | **0.01** | -0.01 | 0.95 |
| Antigen-specific plasma IgG concentration | **0.27** | **0.03** | 0.02 | 0.85 | -0.05 | 0.79 |
| Fc region glycans PC1 | 0.07 | 0.53 | 0.14 | 0.12 | -0.22 | 0.06 |
| Fc region glycans PC2 | **0.26** | **0.01** | -0.08 | 0.55 | 0.07 | 0.65 |

[a] LF: Low frequency
Bolded values indicate statistical significance

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1185  **Figures**

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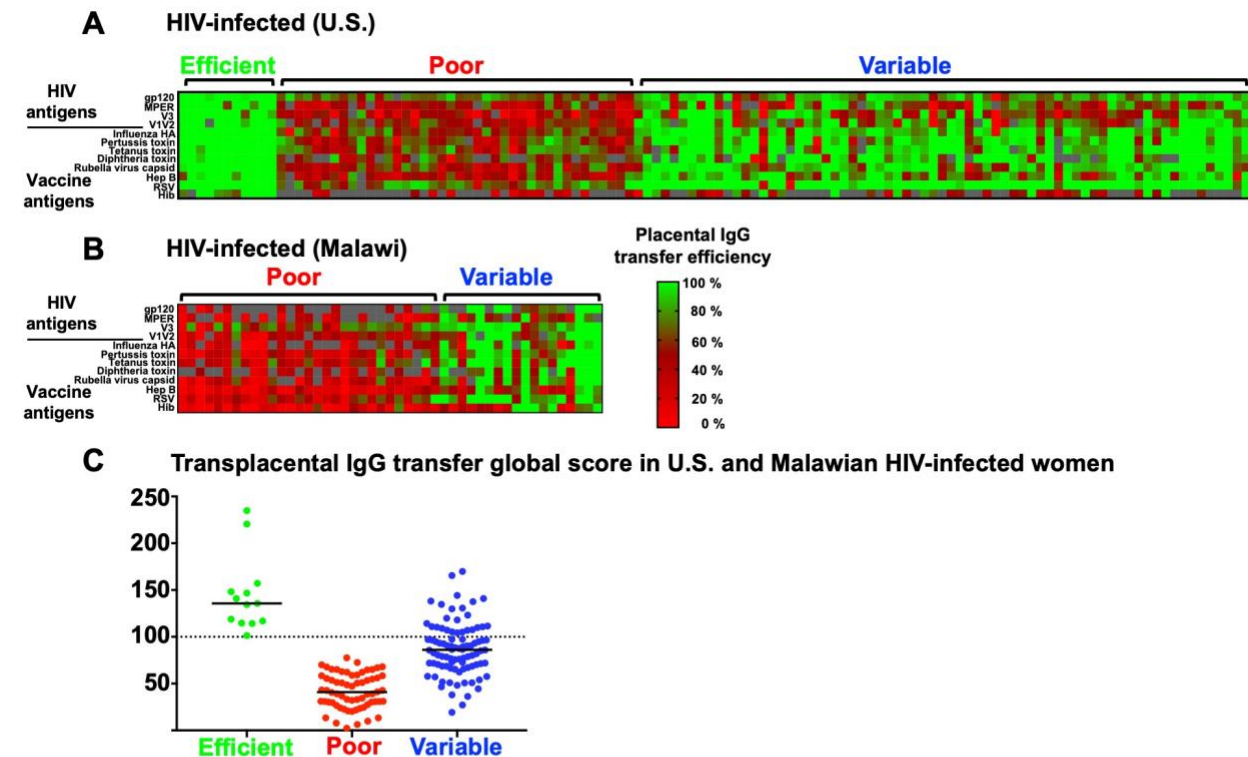


**Figure 1. Distinct phenotypes of transplacental transfer efficiency of HIV and vaccine antigen-specific IgG among HIV-infected pregnant women.** Transplacental IgG transfer efficiency in HIV-infected mother infant pairs from the (A) U.S. and (B) Malawi. (C) Global transplacental IgG transfer score among HIV-infected women defined to have efficient, poor, and variable transplacental IgG transfer. Bar denotes median. Grey squares denote uncalculated transfer ratios from concentrations outside the linear range.

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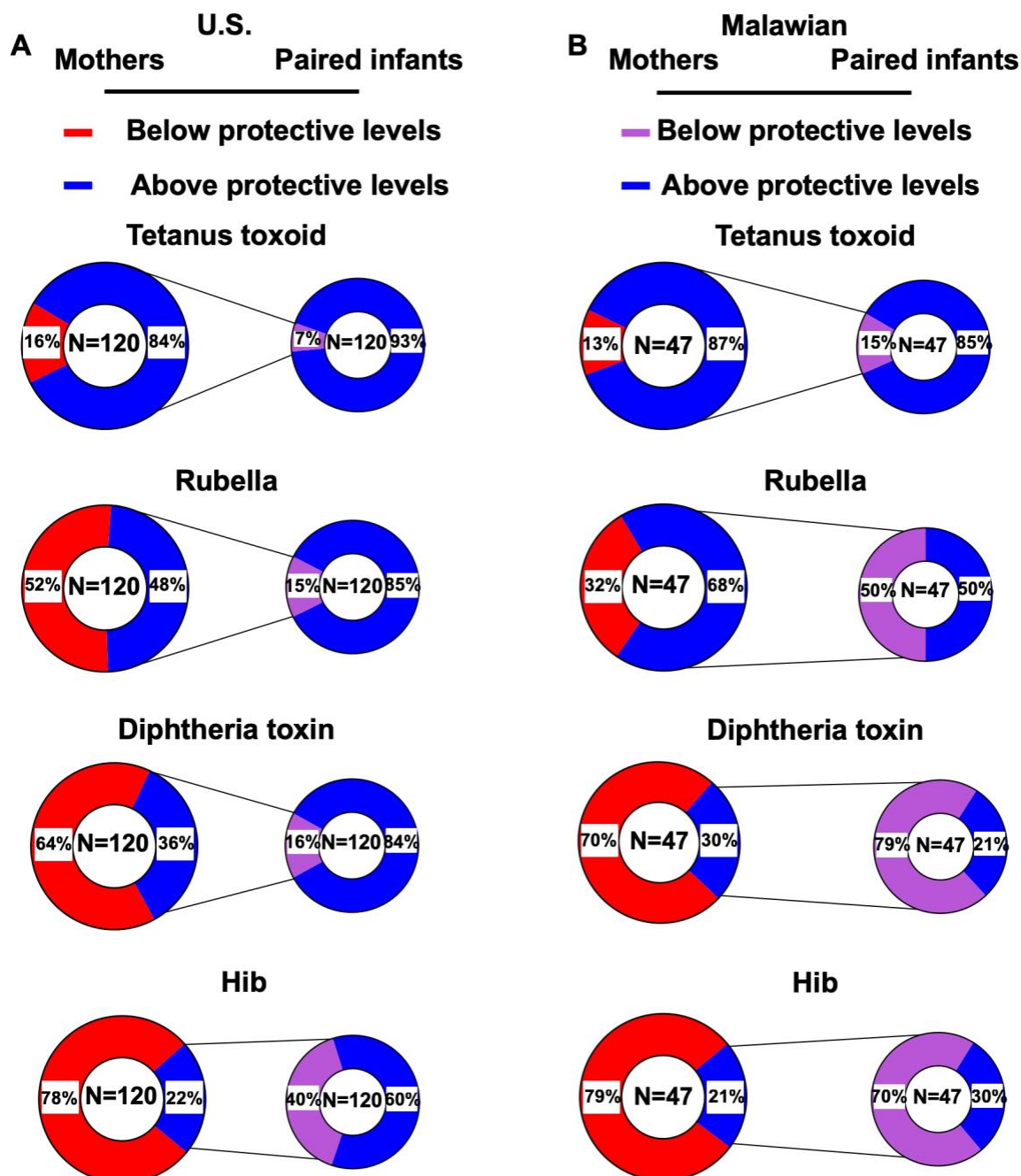
**Figure 2: HEU infants receive suboptimal levels of protective maternal IgG against vaccine-preventable infections.** Proportion of (A) U.S. and (B) Malawian mothers with levels of vaccine-specific IgG below (red) and above (blue) the established protective concentrations against tetanus toxoid, rubella, diphtheria toxin, and *Haemophilus influenzae* type B (Hib). Larger pie graphs show the proportion of mothers with IgG levels above and below the protective concentration. Smaller pie graphs show the proportion of infants born to women with protective IgG levels that fall below (purple) and above (blue) the protective IgG levels at birth.

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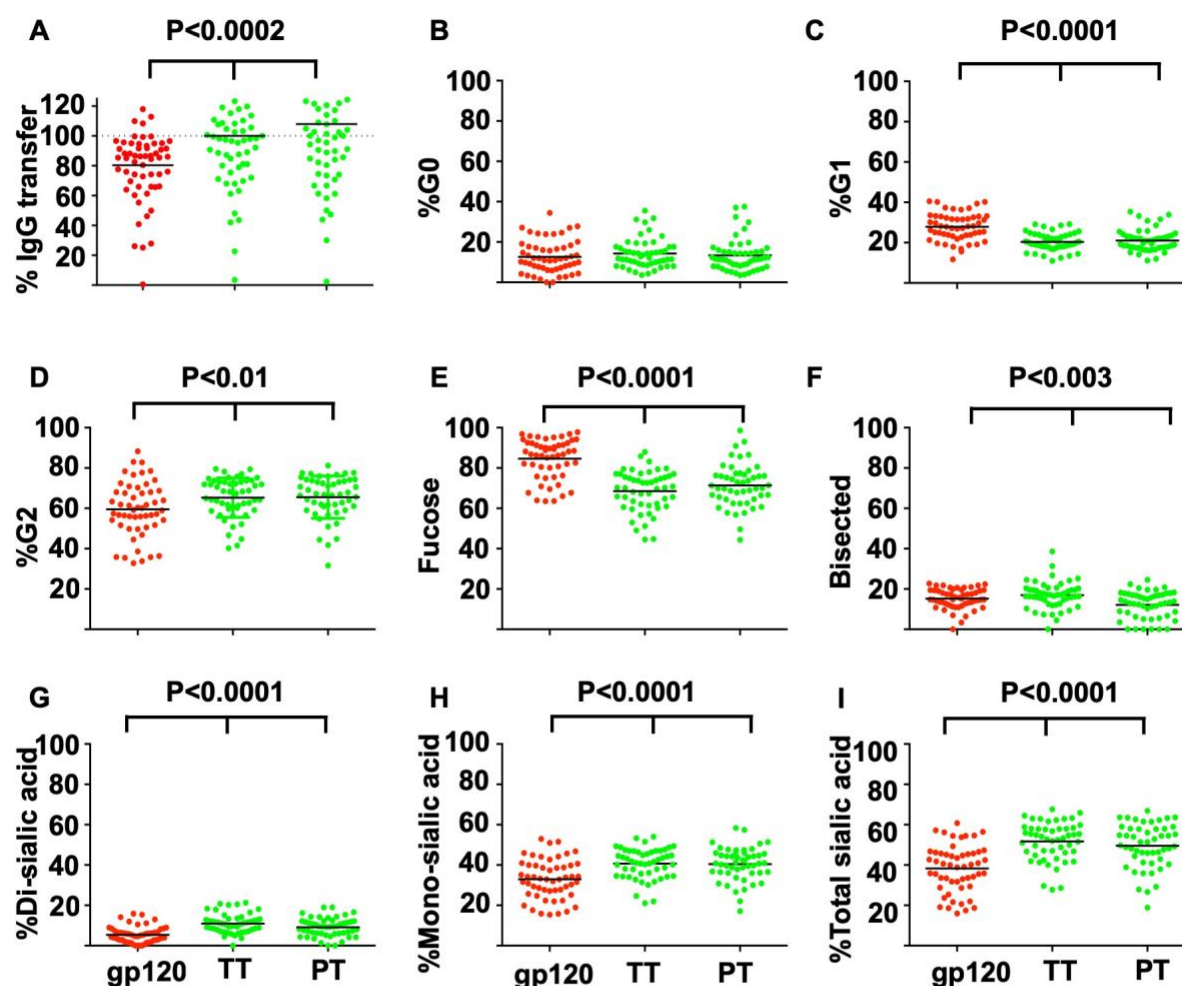


**Figure 3: Differences in Fc region glycan profiles of gp120, tetanus toxoid, and pertussis toxin-specific IgG in U.S. HIV-infected women with variable transplacental IgG transfer.** (A) % transplacental transfer efficiency of gp120, tetanus toxoid (TT), and pertussis toxoid (PT)-specific IgG. Frequency of (B) G0, (C) G1, (D) G2, (E) fucose, (F) bisected, (G) di-sialic acid, (H) mono-sialic acid, (I) total sialic acid of Fc region glycans of gp120, tetanus toxoid, and pertussis toxin-specific IgG. Displayed is p value from a Kruskal Wallis test, dotted line denotes 100% transfer.

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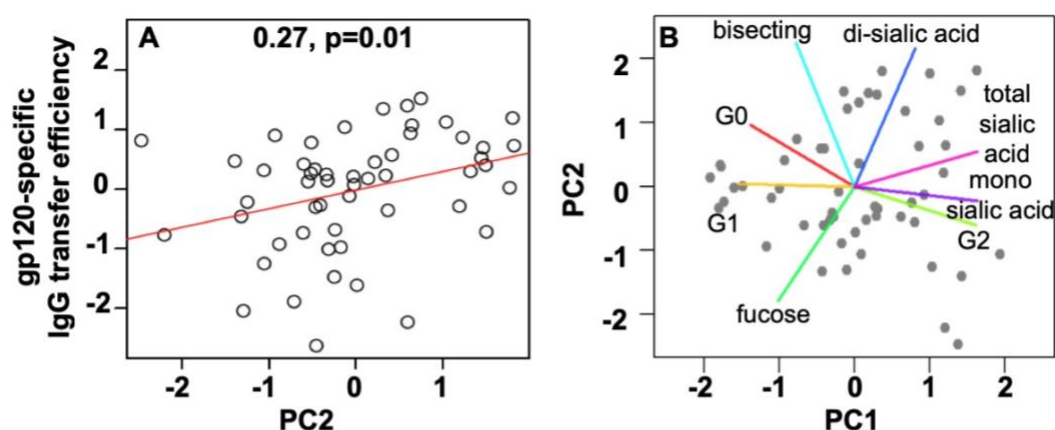


**Figure 4: gp120-specific IgG Fc glycan profile are associated with transplacental IgG transfer efficiency in U.S. HIV-infected women with variable transplacental IgG transfer.** Regression modeling of gp120-specific IgG glycan (A) PC2 (Fucose, Bisecting, and Di-sialic acid) and transplacental IgG transfer efficiency. (B) Fc glycan PC 1 and 2 loadings plot analysis of individual Fc region glycan profiles of gp120-specific IgG.

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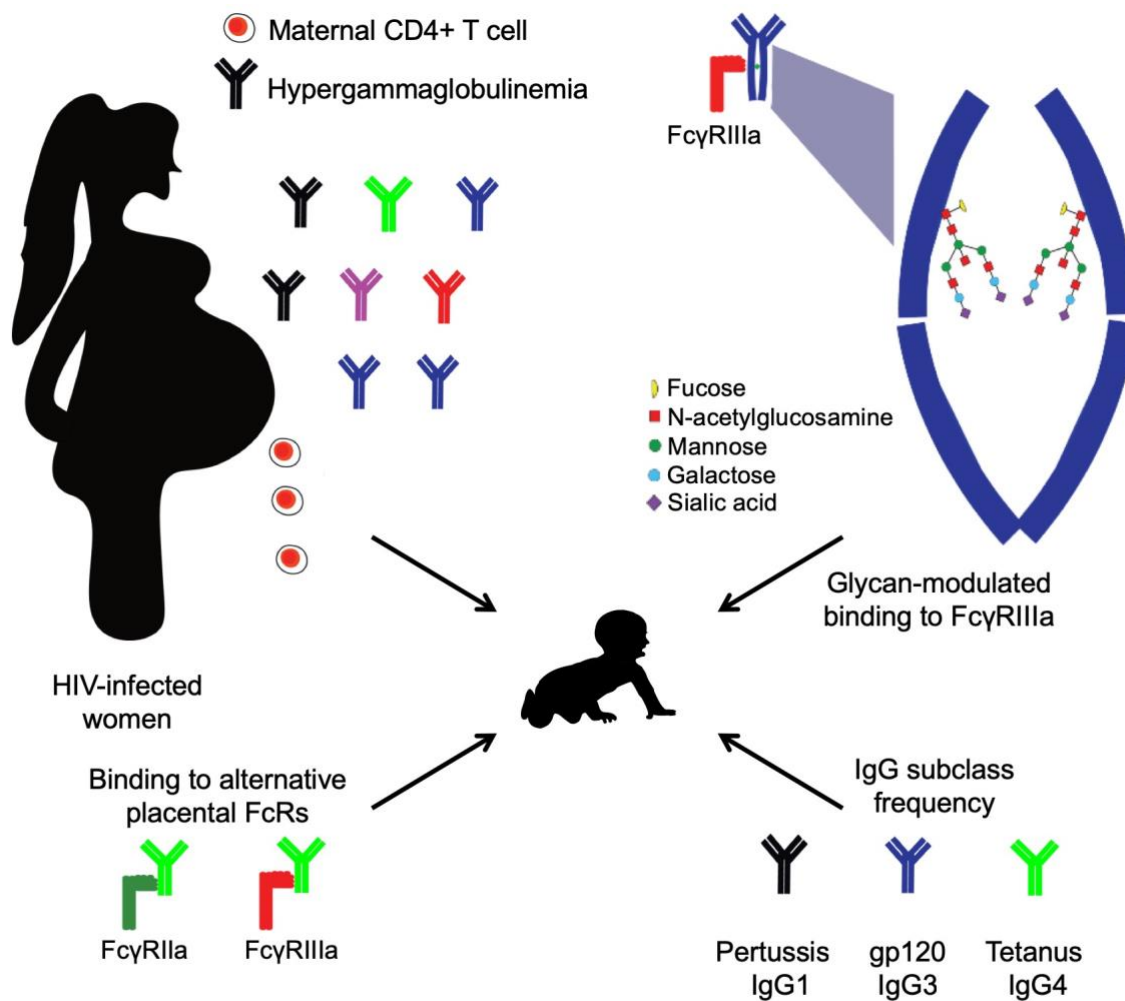


**Figure 5: Model of the selective placental transfer of maternal IgG in HIV-infected pregnant women.**

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1245 **Supplementary Tables and Figures**
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## Table S1. Frequency and concentrations of HIV and non-HIV pathogen-specific IgG response in U.S. and Malawian HIV-infected women

| antigen-specific IgG | U.S. | | | Malawian | | |
|---|---|---|---|---|---|---|
| | frequency | concentration | | frequency | concentration | |
| | | median | range | | median | range |
| HIV gp120 | 99% | 8,181.6 (µg/ml) | 6.5-44,695.6 | 100% | 11,367.7 (µg/ml) | 298.4-24,170.6 |
| HIV MPER | 70% | 2.3 (µg/ml) | 0.01-159.5 | 65% | 1.1 (µg/ml) | 0.03-45.5 |
| HIV V3 | 92% | 19.1 (µg/ml) | 0.02-46,125.4 | 100% | 2,377.6 (µg/ml) | 20.8-3,187.4 |
| HIV V1V2 | 76% | 74 (µg/ml) | 0.3-45,488.6 | 89% | 0.5 (µg/ml) | 0.01-11.9 |
| Influenza HA | 92% | 0.9 (µg/ml) | 0.05-74.6 | 81% | 0.5 (µg/ml) | 0.01-58.1 |
| Pertussis toxin | 89% | 2.9 (IU/ml) | 0.02-110.3 | 96% | 3.2 (IU/ml) | 0.6-48.1 |
| Tetanus toxin | 98% | 0.5 (IU/ml) | 0.0001-5.8 | 98% | 0.7 (IU/ml) | 0.02-6.5 |
| Diphtheria toxin | 92% | 0.05 (IU/ml) | 0.0006-1.5 | 96% | 0.03 (IU/ml) | 0.001-0.4 |
| Rubella virus capsid | 98% | 8.9 (IU/ml) | 0.6-261.1 | 98% | 17.2 (IU/ml) | 0.7-186.6 |
| Hep B | 79% | 1.9 (IU/ml) | 0.03-33.0 | 96% | 3.1 (IU/ml) | 0.1-36.5 |
| RSV | 99% | 8.4 (µg/ml) | 0.1-57.9 | 100% | 17.2 (µg/ml) | 2.71-65.1 |
| Hib | 56% | 0.03 (µg/ml) | 0.00002-15.2 | 70% | 0.03 (µg/ml) | 0.001-6.5 |

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## Table S2. Antigen-specific IgG glycan Principal Components (PC) percent variance in U.S. HIV-infected women with variable transplacental IgG transfer
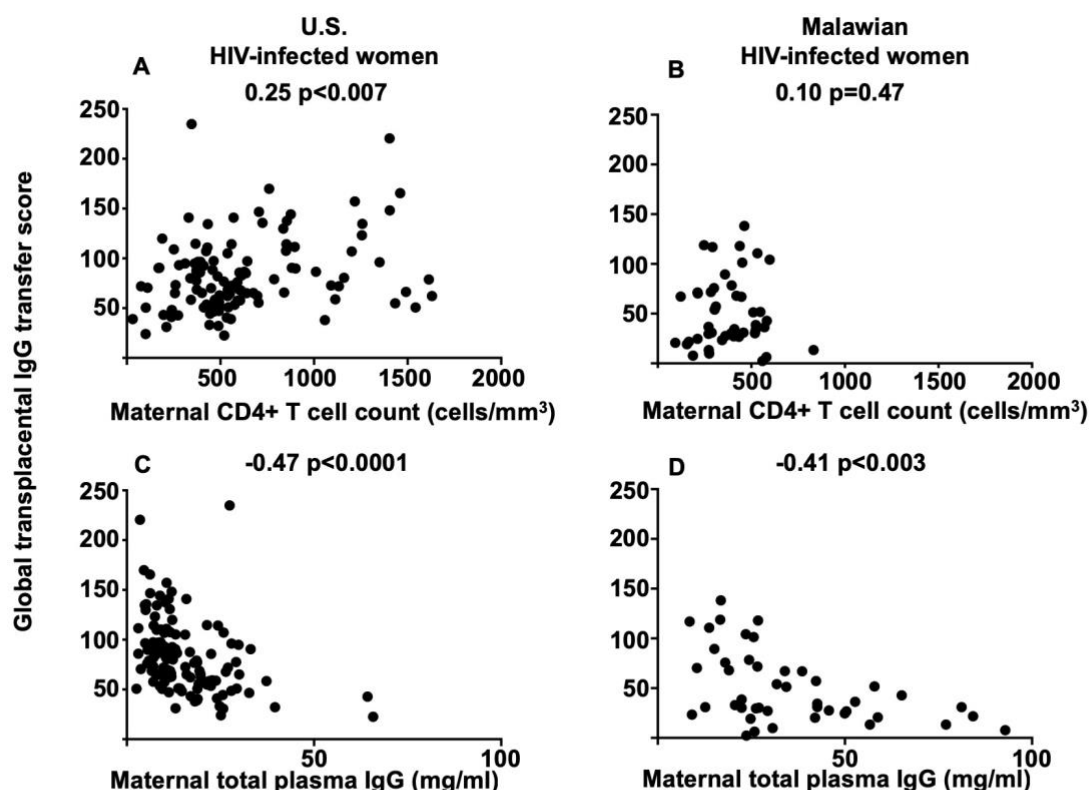
| IgG Fc Glycan Components | gp120-specific IgG | tetanus toxoid-specific IgG | pertussis-toxin-specific IgG |
|---|---|---|---|
| **PC 1**: G0, G1, G2, mono-sialic acid, and total sialic acid | 64[a] | 62 | 59 |
| **PC 2**: fucose, disialic acid, and bisected | 24 | 16 | 19 |
| PC 3 | 5 | 11 | 11 |
| PC 4 | 4 | 7 | 7 |
| PC 5 | 2 | 3 | 2 |
| PC 6 | 1 | 0 | 2 |
| PC 7 | 0 | 0 | 0 |
| PC 8 | 0 | 0 | 0 |

[a] % variance
Bolded values indicate variance >20%

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**Figure S1: Maternal CD4+ T cell counts and total plasma IgG concentrations and transplacental IgG transfer efficiency.** Correlation of maternal CD4+ T cell counts in A) U.S. and B) Malawian HIV-infected women and transplacental IgG transfer efficiency. Correlations of maternal total plasma IgG concentrations in C) U.S. and D) Malawian women and transplacental IgG transfer efficiency. Spearman correlation and p values are shown.

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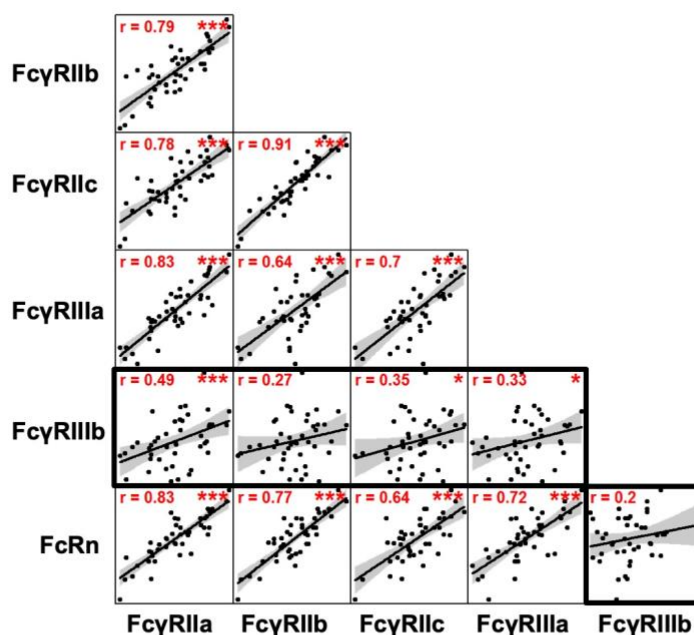
**Figure S2: Strong correlations between the levels of mRNA expression of FcRs in placental tissues of HIV-infected Malawian women.** Fc receptor expression levels in the placenta highly correlated between FcRn, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa (r: 0.64-0.91), yet there was a low correlation of FcγRIIIb and other FcRs (r: 0.2-0.49, bolded boxes) in the placenta. Line of best fit is shown and the 95% pointwise confidence interval is shown in grey. * denotes medium correlation and *** denotes strong correlation.

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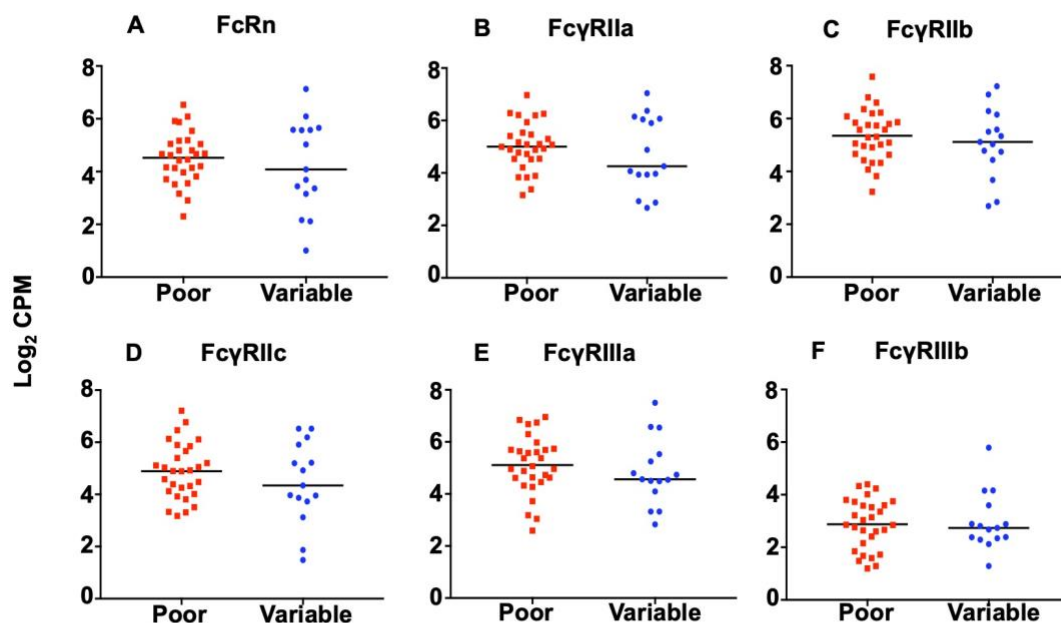


**Figure S3: No difference in placental Fc receptor expression levels in HIV-infected Malawian women with distinct transplacental IgG transfer phenotypes.** Placental mRNA expression levels of (A) FcRn, (B) FcγRIIa, (C) FcγRIIb, (D) FcγRIIc, (E) FcγRIIIa, and (F) FcγRIIIb in HIV-infected women with poor transplacental IgG transfer efficiency (red squares) and variable transplacental IgG transfer efficiency (blue circles). $Log_2$CPM denotes the counts per million of RNA copies. All comparisons of FcR expression between women with poor and variable placental transfer were not statistically significant (raw $p > 0.05$).

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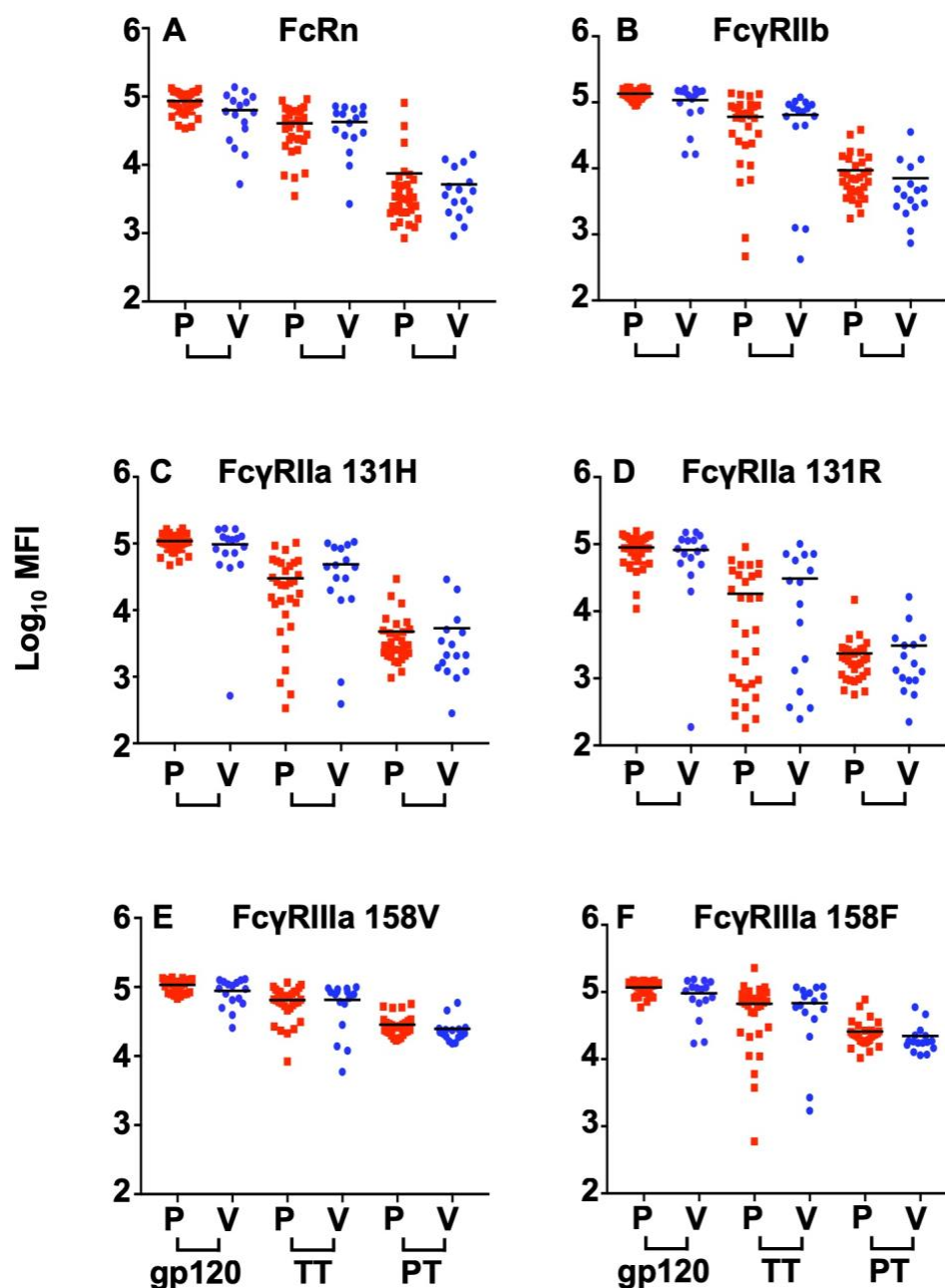
**Figure S4: HIV gp120, tetanus toxoid, and pertussis toxin-specific IgG binding to placental FcRs in HIV-infected Malawian women with poor (P) and variable (V) placental IgG transfer.** Maternal gp120, tetanus toxoid (TT), and pertussis toxin (PT)-specific IgG binding to (A) FcRn, (B) FcγRIIb, (C) FcγRIIa 131H, (D) FcγRIIa 131R, (E) FcγRIIIa 158V, and (F) FcγRIIIa 158F. FcγRIIa 131H and FcγRIIIa 158V are high affinity IgG binding FcRs, whereas FcγRIIa 131R and FcγRIIIa 158F are lower affinity IgG binding FcR polymorphisms. HIV-infected women with poor (P) transplacental IgG transfer efficiency are shown in red squares and variable (V) transplacental IgG transfer efficiency in blue circles. $Log_{10}$ MFI denotes the mean fluorescent intensity.

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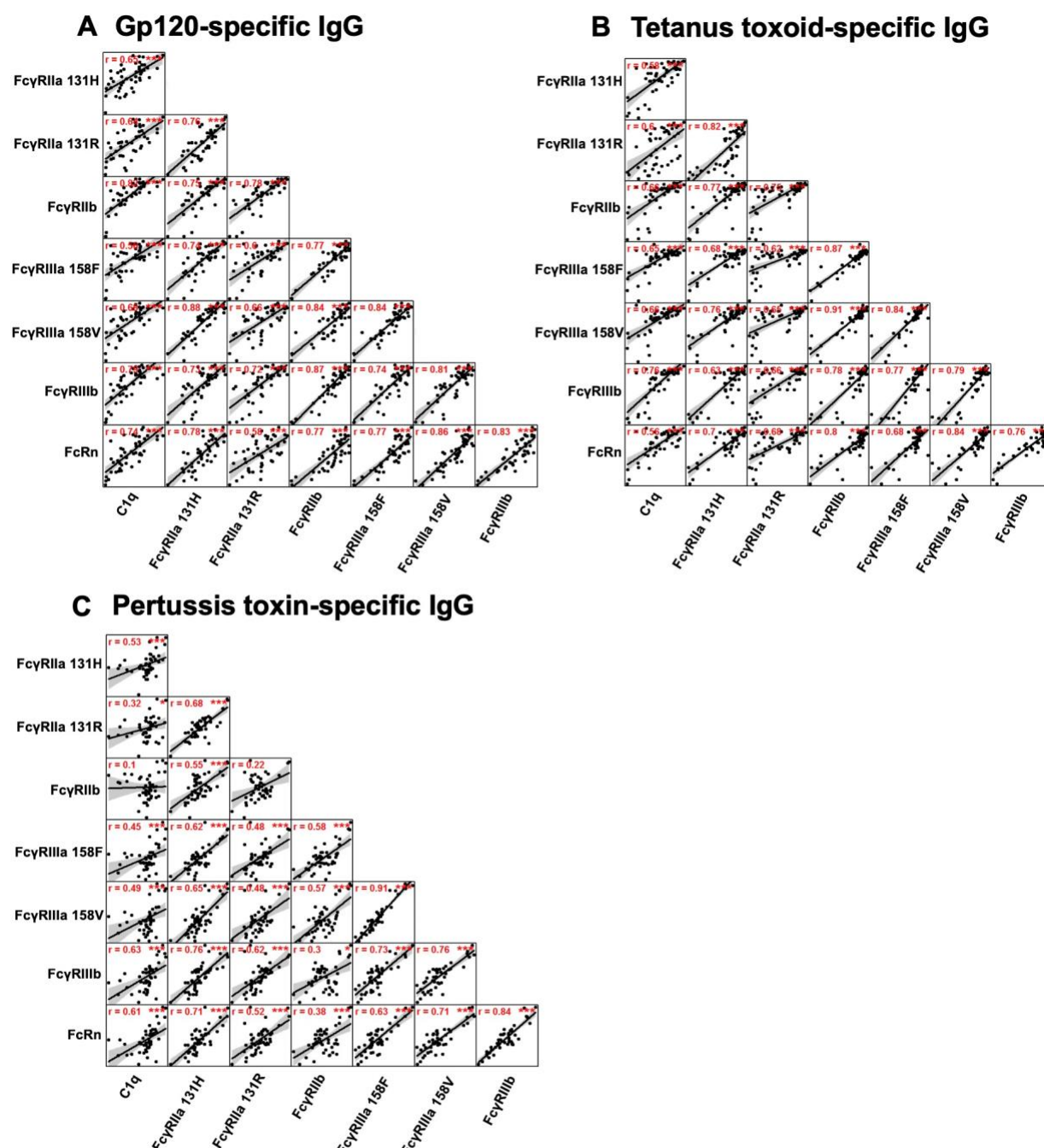


**Figure S5: Correlations of binding magnitude of gp120, tetanus toxoid, and pertussis-specific IgG to distinct placental FcRs.** Correlation of maternal gp120, tetanus toxoid, and pertussis toxin-specific IgG binding to C1q and placentally expressed FcRn, FcγRIIb, FcγRIIa 131H, FcγRIIa 131R, FcγRIIIa 158V, FcγRIIIa 158F, FcγRIIIb in HIV-infected Malawian women. Line of best fit is shown and the 95% pointwise confidence interval is shown in grey.

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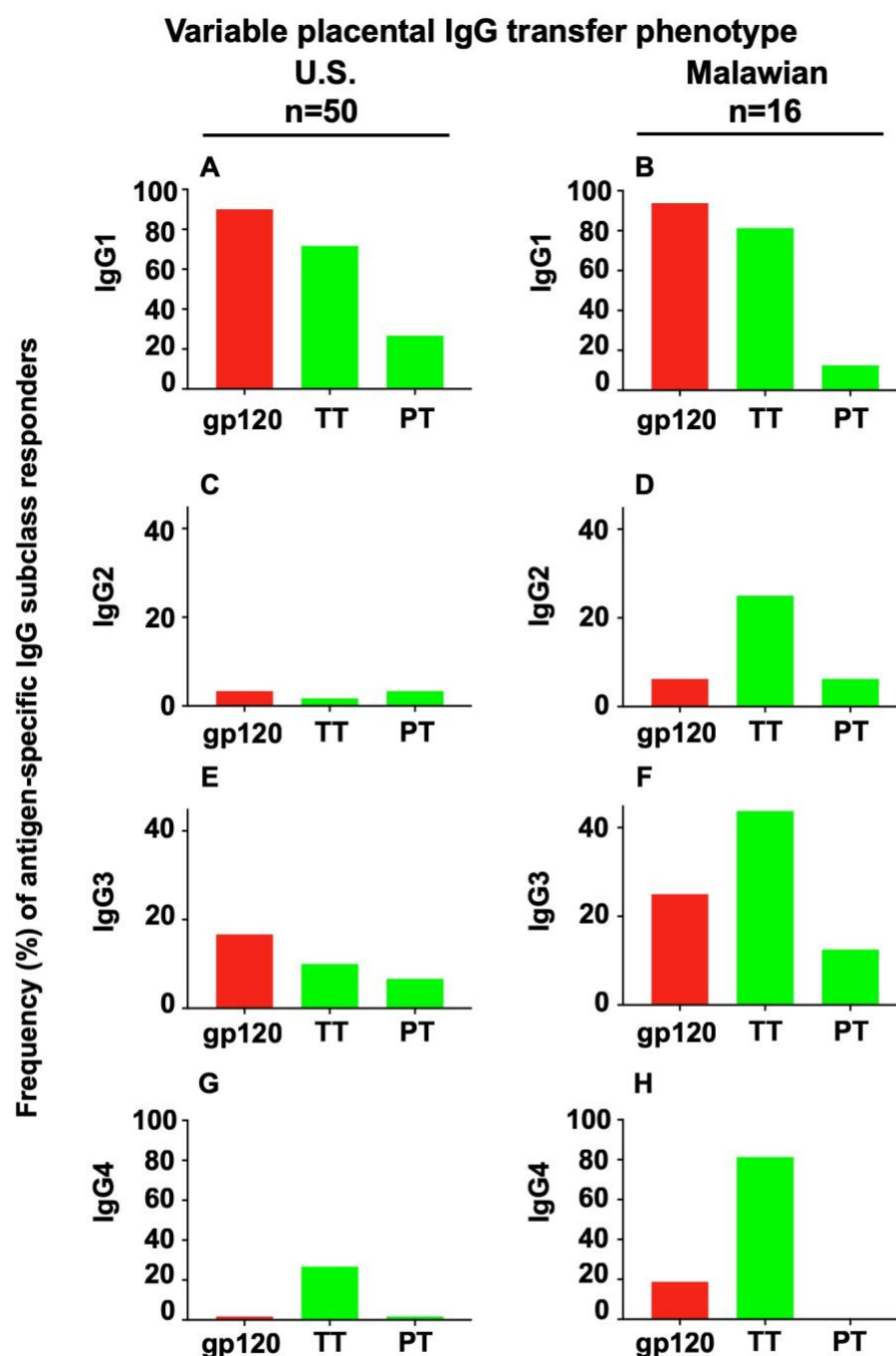


**Figure S6: Frequency of gp120, tetanus toxoid (TT), and pertussis toxin (PT)-specific IgG subclass responders among HIV-infected, U.S. and Malawian women with variable transplacental IgG transfer.** Frequency of plasma gp120, tetanus toxoid (TT), and pertussis toxin (PT)-specific IgG1 (A, B), IgG2 (C, D), IgG3 (E, F), IgG4 (G, H) subclass responses in U.S. (A, C, E, G) and Malawian (B, D, F, H) women with variable transplacental IgG transfer.