IgG antibodies to dengue enhanced for FcγRIIIA binding determine disease severity

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Dengue virus (DENV) infection in the presence of reactive, non-neutralizing immunoglobulin G (IgG) (RNNIg) is the greatest risk factor for dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Progression to DHF/DSS patients respond to infection by producing IgGs with enhanced affinity for the activating Fc receptor FcγRIIIA due to afucosylated Fc glycans and IgG1 subclass. RNNIg enriched for afucosylated IgG1 triggered platelet reduction in vivo and was a significant risk factor for thrombocytopenia. Thus, therapeutics and vaccines restricting production of afucosylated, IgG1 RNNIg during infection may prevent ADE of DENV disease.

Antibody-dependent enhancement (ADE) has been shown to occur in a variety of in vitro and in vivo dengue virus (DENV) infection models, but ADE does not explain why fewer than 15% of human DENV infections that occur in the presence of reactive, non-neutralizing immunoglobulin G (IgG) (RNNIg) progress to dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Progression to DHF/DSS patients advance to DHF/DSS, the presence of RNNIg alone cannot account for disease severity. We discovered that DHF/DSS patients respond to infection by producing IgGs with enhanced affinity for the activating Fc receptor FcγRIIIA due to afucosylated Fc glycans and IgG1 subclass. RNNIg enriched for afucosylated IgG1 triggered platelet reduction in vivo and was a significant risk factor for thrombocytopenia. Thus, therapeutics and vaccines restricting production of afucosylated, IgG1 RNNIg during infection may prevent ADE of DENV disease.
Fig. 1. Afucosylated Fc glycoforms and IgG1 subclass are enriched in dengue infection. (A) Anti-ENV IgGs in early DENV or convalescent DENV infection (conv) show an increased abundance of afucosylated Fc glycans (afucFc) compared with healthy adults (uninfected). IgGs on day 7 after IAV infection (IAV) or Octagam or Flebogam intravenous immunoglobulin (IVIG) preparations. (B) No difference among sialylated Fc glycoforms (sFc) was observed. (C) Anti-ENV IgGs were skewed in distribution toward the IgG1 subclass during early or convalescent DENV infection compared with anti-HA IgGs from uninfected healthy adults, patients on day 7 of IAV infection, or Octagam or Flebogam IVIG preparations. (D) DENV patient IgGs that were reactive with DENV NS1 protein, cross-reactive/reactive with Zika virus ENV, or with HA protein were also elevated in afucFc relative to anti-HA IgG from IAV patients. (E) Zika ENV-reactive IgGs in early DENV infection were skewed in distribution toward the IgG1 subclass.

Fig. 2. Activating Fc phenotype in dengue infection correlates with disease severity. (A) Increased afucFc on anti-ENV or anti-HA IgG correlated with DHF/DSS (DHF). (B) Stronger correlations yet were observed when patients were stratified based on thrombocytopenia (TH+) (platelets <100,000/ul) during hospitalization. TH+ patients had elevated abundance of afucFc on anti-ENV or anti-HA IgG compared with TH− patients. (C) TH+ patient anti-ENV IgGs were skewed in distribution toward the IgG1 subclass, whereas anti-HA IgGs from TH+ patients were not. (D and E) Patients with the lowest recorded platelet count during hospitalization had the greatest abundance of afucFc and the highest IgG1/IgG2 ratio of anti-ENV IgGs. (F) Abundance of afucFc on anti-ENV IgG correlated with IgG1/IgG2 distribution; elevated afucFc and IgG1/IgG2 correlated with the severity of thrombocytopenia. The single patient with DSS in the cohort had the greatest abundance of afucFc and the highest IgG1/IgG2 ratio (marked with *). (G and H) Elevated hematocrit (HCT), a marker of plasma leak that distinguishes DHF/DSS from DF, correlated with anti-ENV IgGs having the greatest abundance of afucFc and the highest IgG1/IgG2 ratio.
elevated in afucFc compared with anti-HA IgG from IAV patients (Fig. 1D), indicating that a global shift in IgG Fc structure had occurred in early DENV infection. In addition, IgGs reactive with the ENV protein of the infecting DENV serotype, or reactive with ENV proteins from the noninfecting DENV serotypes, were equivalent in abundance of afucFc in a subgroup analysis (fig. S1). Zika ENV-reactive antibodies were also elevated in IgG1/IgG2 ratio, whereas IgGs reactive with DENV NS1 or IAV HA proteins were not (Fig. 1E), indicating that modulation of Fc fucosylation was a more general feature in DENV infection than subclass bias.

Stratification of patients by clinical diagnosis showed that anti-ENV and anti-HA IgGs from DHF/DSS patients were elevated in afucFc compared with IgGs from either DF or IAV patients (Fig. 2A). Stratification of patients based on presence of thrombocytopenia (TH+) during disease course, which is a requisite criterion for DHF diagnosis, showed that TH+ patients had similarly elevated afucFc anti-ENV and anti-HA IgGs, with afucFc ≥ 10% being a significant risk factor for TH+ (P = 0.0139; odds ratio 11.00; 95% confidence interval 1.635 to 74.00; relative risk 1.833) (Fig. 2B). TH+ also correlated with an increased IgG1/IgG2 ratio for anti-DENV ENV (Fig. 2C). Total IgG from TH+ patients had substantially higher affinity for FcRIIA by surface plasmon resonance (fig. S2).

Elevated afucFc and IgG1/IgG2 ratio not only correlated with being TH+ during hospitalization but also correlated with the lowest platelet count recorded for each patient during hospitalization (Fig. 2, D and E). Further, these two determinants of higher-affinity binding to FcRIIIA correlated with each other, and patients with the greatest abundance of afucFc IgG1 were most likely to have severe thrombocytopenia (Fig. 2F).

The single patient with DSS had the greatest abundance of afucFc IgG1 of all patients in the cohort (Fig. 2F). In addition to clinical diagnosis and severity of TH+, elevated hematocrit, an indication of plasma leak that distinguishes DHF/DSS from DF, also correlated with the abundance of afucFc and the elevated IgG1/IgG2 ratio of anti-ENV IgGs (Fig. 2, G and H).

The correlation between high afucFc IgG1 and severe disease indicated that this Fc structure may play a role in ADE during DENV infection. In particular, the correlation between the abundance of afucFc IgG1 and the degree of thrombocytopenia (Fig. 2, D to F) led us to hypothesize that anti-DENV IgGs that cross-react with platelet antigens might contribute to platelet loss during dengue infection and thus to ADE of dengue disease. Because anti-DENV NS1 IgG has been shown to cross-react with platelets (37), we tested whether transfer of IgG from severely TH+ patients could mediate platelet reduction, in vivo, to a greater extent than IgG from TH− patients. IgGs from TH+ patients caused a drop in platelets in mice humanized for FcRs (hFcR), whereas, even at high doses, IgG from TH− patients did not reduce platelet numbers (fig. S3).

IgG from TH+ patients was treated to remove Fc glycans, producing an aglycosylated TH+ pool (TH+ agly) that would no longer engage FcRs; this pool had less effect on platelet numbers, whereas mice lacking all FcRs (α−/−) were resistant to TH+ IgG-mediated thrombocytopenia (Fig. 3A) (38). This loss of platelets was dependent on two low-affinity human activating FcRs, FcγRIIA (CD32A) and FcγRIIIA (CD16A), because deletion of either receptor rendered the mice resistant to TH+ IgG-induced platelet reduction (Fig. 3B).

Because purified IgG alone caused a loss of platelets, we next investigated whether IgG from TH+ patients might bind to platelets directly. After incubation with human or mouse platelets, TH+ IgG could be eluted from platelets that bound the DENV NS1 protein but not the ENV protein (Fig. 3C and fig. S4). As with anti-ENV IgG from TH+ patients, anti-NS1 IgG had elevated afucFc (Fig. 3D), but the IgG1/IgG2 ratio was not different (fig. S5). All together, this showed that the IgGs enhanced for FcγRIIIA binding from patients who became thrombocytopenic during DENV infection could mediate FcR-dependent platelet loss in vivo. At least three mechanisms could contribute to this platelet reduction: anti-NS1/platelet IgG may activate platelets directly through platelet FcγRIIIA, and/or cause sequestration or uptake of platelets by monocytes that express both FcγRIIIA and FcγRIIIA, and/or ADCC of platelets could occur via FcγRIIIA (38).

These experiments showed that anti-DENV IgGs with enhanced affinity for FcγRIIIA could mediate ADE of disease, which is distinct from ADE of infection. Serum pools of both TH+ and TH− patients mediated ADE of DENV infection in the standard U937 cell assay (fig. S6). This result, while confirming that IgGs from RNNIg+ DENV patients can mediate ADE, is not informative in the context of our observations that
enhanced dengue disease is associated with an FcRIIIA-activating IgG phenotype. This is because U937 cells are FcγRIIA, FcγRIIA⁺ (38–41). Thus, we distinguish between ADE of infection and ADE of disease in our study.

That the precise Fc structure of antibodies present during DENV infection may contribute to disease severity raised the question of whether this Fc structure was present before infection or was triggered by DENV infection itself. To address this, we compared the Fc of antibodies obtained from TH⁺ patients during the early and convalescent phases of disease. The convalescent phase was marked by a significant drop in both afucFc and IgG1/IgG2 ratio (Fig. 4, A and B), indicating that, in patients with severe disease, DENV infection itself triggered an elevation in IgGs with enhanced affinity for FcγRIIIA.

The present finding that some individuals respond to DENV infection by producing IgGs with higher affinity for FcγRIIIA indicates a host determinant of susceptibility to severe DENV disease. Further studies will determine how this patient selectivity may contribute to additional mechanisms underlying ADE of DENV disease.

REFERENCES AND NOTES
34. Materials and methods are available online as supplementary materials.
41. See the methods online.

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SUPPLEMENTARY MATERIALS
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Materials and Methods
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**Editor's Summary**

**A rare ability to enhance dengue virus disease**

In some cases, secondary infections of dengue virus can be extremely serious and result in plasma leakage, thrombocytopenia, and hemorrhagic disease. This phenomenon has been attributed to antibody-dependent enhancement. Wang et al. show that a specific subclass of antibody, IgG1, which lacks fucosyl residues on the Fc segment of the heavy chain of the immunoglobulin, is elevated in patients with severe secondary dengue disease. These non-neutralizing antibodies bind activating Fc receptors and appear to cross-react with platelet antigens to cause platelet depletion, contributing to thrombocytopenia.

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