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SUPPORTING INFORMATION

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IgE glycosylation is essential for the function of omalizumab

To the Editor,

Omalizumab, a humanized anti-IgE antibody, is the only currently approved antibody for treating IgE-mediated diseases and acts by interfering with IgE binding to the IgE receptors FcεRI and CD23 (FcεRII).[1]

Shade et al. demonstrated that IgE sialylation is essential for its allergic potential.[2] The introduction of a glycan structure on IgE at the binding site for omalizumab prevents the binding of omalizumab to IgE.[3] We have demonstrated that natural anti-IgE antibodies in mice and humans recognize glycan structures on IgE.[4,5] However, it is not known whether the removal of glycan structures also affects the activity of omalizumab.

To study this, we deglycosylated human IgE antibodies obtained from three different sources, two from hybridomas (SUS11

FIGURE 1 Reduced binding of omalizumab to enzymatically deglycosylated human IgE and recombinant mutated IgE. (A) The results of ELISA OD450 of omalizumab binding to glycosylated or deglycosylated human IgE antibodies are shown. Binding of omalizumab to isolated IgE from human serum compared with human IgE treated with Endoglycosidase F1 ($n=4$ per group). Binding kinetics of glycosylated (B) and deglycosylated (C) human IgE to omalizumab in BLI assays. In all assays, both association and dissociation were performed for 300 s. (D) Comparison of binding of glycosylated versus deglycosylated IgE to CD23. IgE antibodies and omalizumab at different concentrations were complexed before they were added to the B cells. Shown are isolated human IgE treated with Endoglycosidase F1 ($n=3$ per group). (E) shows the introduction of mutation in the Cε3 region. (F) shows the ELISA OD450 values of omalizumab binding to WT recombinant IgE or mutated IgE ($n=6$ per group). (G) shows the response of omalizumab to recombinant, and (H) shows the response of omalizumab to mutated IgE in BLI. (I) Comparison of binding of glycosylated versus Endo F1 treated isolated human IgE to CD23 ($n=3$ per group). IgE antibodies and omalizumab at different concentrations were complexed before they were added to the B cells. Statistical analysis for (A, D, F), and (I) was performed using ordinary two-way ANOVA with Šidák's multiple comparison test ($a=0.05$); ns = not significant, $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$ and $^{****}p \leq 0.0001$.

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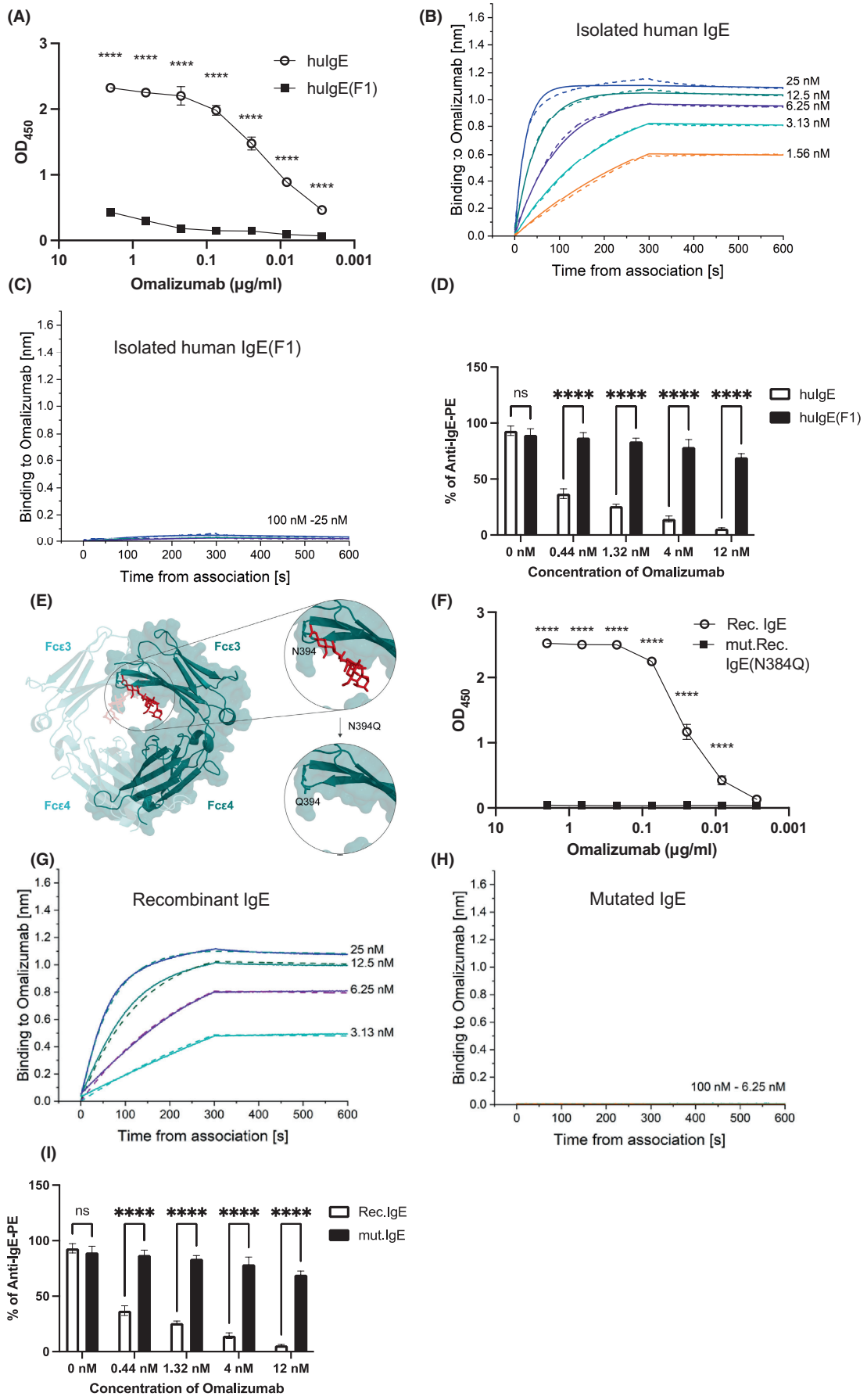

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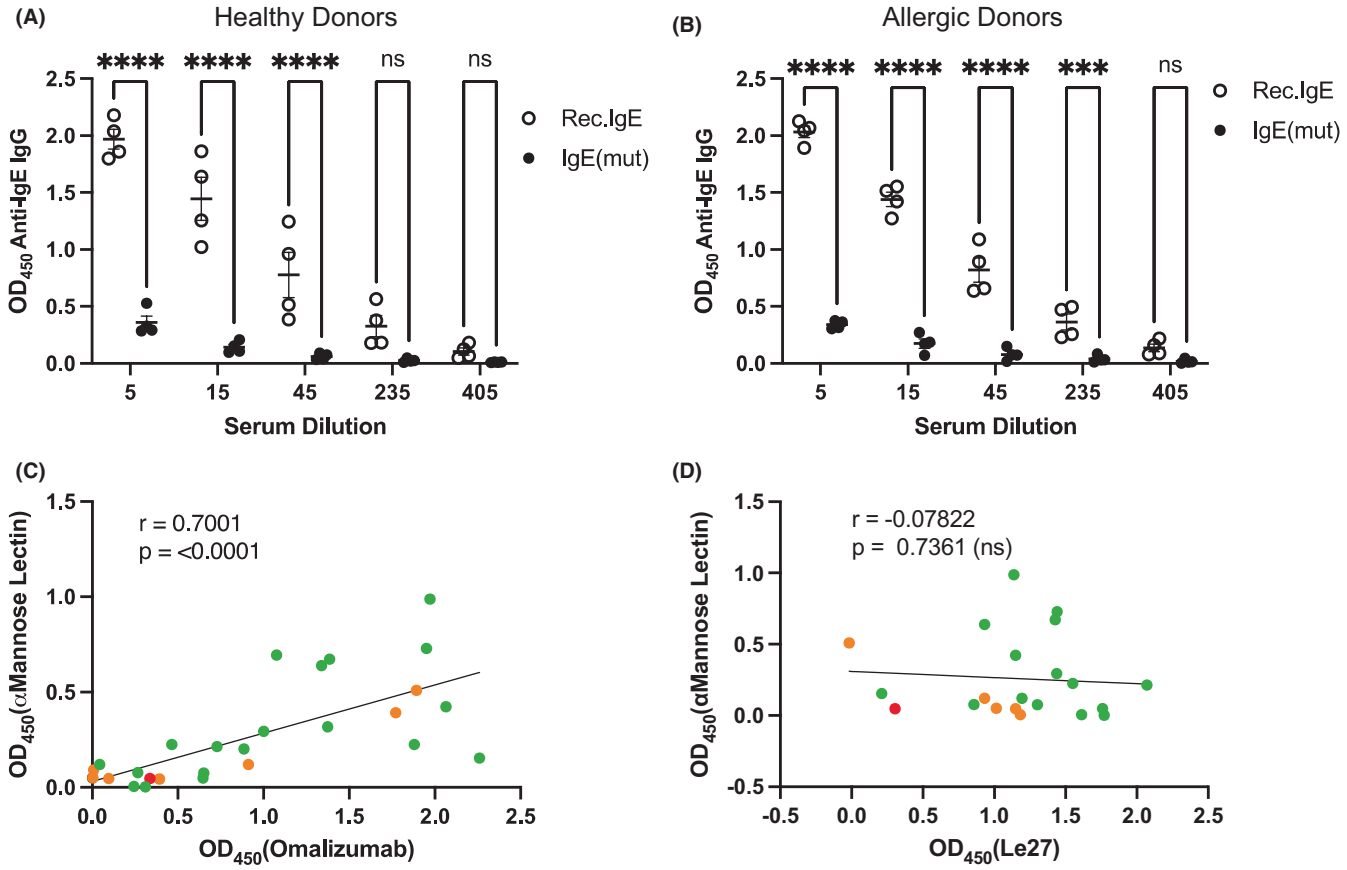

FIGURE 2 Assessment of glycan binding by human serum and Omalizumab. The results of ELISA OD450 of human sera from healthy (A) or atopic (B) individuals binding to recombinant or mutated human IgE antibodies are shown. (C, D) shows the scatter plot of the OD450 value ($n \geq 21$). Shown is the correlation of atopic individuals to anti-mannose lectin and omalizumab (C) or anti-mannose lectin and control anti-IgE antibody Le27 D). The color of the dots represents the amount of total IgE: green >100 kU/mL, orange 20–100 kU/mL, red <20 kU/mL. (B, D) were analyzed using ordinary two-way ANOVA with Šidák's multiple comparison test ($a = 0.05$). (C and D) were analyzed using the Pearson correlation coefficients (two-tailed) with a confidence interval of 95%.

and ab65866) and IgE isolated from human serum. As a single oligomannose-type glycan is essential for binding to the FcεRI,[6] we used Endoglycosidase F1 to remove the oligomannose at the Cε3 domain resulting in a substantial reduction in binding to deglycosylated IgE compared with glycosylated IgE (Figure 1A and Figure S1A,B).

Removing oligomannose from IgE not only reduced the binding ability of omalizumab to IgE but also decreased the binding constants of omalizumab to IgE as determined in Biolayer Interferometry (BLI) measurements (Figure 1B,C and Figure S1C–G).

Given that CD23, unlike FcεRI, binds IgE independent of its glycosylation, we examined whether the removal of oligomannose may affect the inhibition of IgE binding to CD23 by omalizumab on CD23 expressing B-cell line. Significantly, better inhibition was observed with glycosylated IgE (Figure 1D and Figure S1H,I). However, there was a different extent of inhibition depending on the IgE source. Interestingly, when we mutated (N394 to Q394) the recombinant IgE, omalizumab could no longer bind this mutant IgE (Figure 1E,F), confirming that the loss of binding of omalizumab to deglycosylated IgE is due to the absence of N394 glycosylation. Likewise, no binding of omalizumab to mutated IgE could be detected in BLI (Figure 1G,H) and no blocking of IgE binding to CD23 could be observed (Figure 1I).

Since glycosylation of IgE seems to play a significant role in omalizumab's activity, we next investigated the role of various IgE glycans in the activity of omalizumab in more detail. First, we examined whether the removal of sialic acid affects IgE binding since healthy individuals and peanut-allergic individuals show different sialic acid patterns.[2] Our data showed that removing the sialic acid from complex and hybrid N-glycans did not significantly affect omalizumab binding to IgE, which corroborates the oligomannose specificity of omalizumab binding (Figure S2A). However, our glycan arrays showed that omalizumab could not recognize glycan structures independently of the IgE protein backbone (Figure S2B,C), suggesting a conformational change of IgE in the absence of site-specific glycosylation may be responsible for the reduced activity of omalizumab.

Our data may explain why not all allergic patients respond equally well to omalizumab treatment. Indeed, omalizumab responders had significantly higher expression levels in clusters of genes responsible for regulating mannose metabolism than non-responders.[7] Using mutated

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recombinant IgE, we were able to show that natural anti-glycan IgG antibodies comparable to omalizumab are also present in the serum of allergic and healthy individuals (Figure 2A,B). We also observed a significant positive correlation ($r > 0.7$) between the binding of anti-mannose lectin and omalizumab to IgE, whereas no correlation could be detected using a control anti-human IgE antibody, Le27 (Figure 2C,D). These data show that the signal to oligomannose positively correlates with the binding of omalizumab. Differences in oligomannose content on IgE could therefore be a reason for non-responders as well as a marker for the success of omalizumab therapy. However, further studies with more donors and different allergies must confirm our results.

AUTHOR CONTRIBUTIONS

KP, LM, SVG, PE, MFB, and MV designed experiments, acquired data, interpreted data, and analyzed data. GSA, MV, and KP designed the vector of the mutated IgE, expressed, and produced proteins. LJ provided serum of allergic volunteers. KP, LM, SVG, LJ, MFB, PE, and MV wrote and revised the manuscript. MV and MFB supervised the study. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

M. F. Bachmann is a board member of Saiba AG. All other authors declare no conflict of interest.

KEYWORDS

allergy, glycans, hypersensitivity, IgE regulation, omalizumab

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