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Title: IgE glycosylation is essential for the function of omalizumab

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To the Editor

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Omalizumab, a humanized anti-IgE antibody, is the only currently approved antibody for treating IgEmediated diseases and acts by interfering with IgE binding to the IgE receptors FccRI and CD23 (FccRII)(1).

Shade et al. demonstrated that IgE sialylation is essential for its allergic pathogenicity(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 and ab65866) and IgE isolated from human serum. As a single oligomannose-type glycan is essential for binding to the $Fc\epsilon RI(6)$, we used Endoglycosidase F1 to remove the oligomannose at the C ϵ 3 domain resulting in a substantial reduction in binding to deglycosylated IgE compared to glycosylated IgE (Figure 1A and Figures 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 and Figures S1C-G).

Given that CD23, unlike FccRI, 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 Figures 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 (Figures 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 (Figures 1G, H) and in CD23-expressing cells (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 peanutallergic 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 (Figures 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 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. (Figures 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 (Figures 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.

Keywords: Omalizumab, glycans, IgE regulation, allergy, hypersensitivity,

Conflict of interest

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

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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 to 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 in 300s. 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 C ϵ 3 region. F) shows the ELISA OD450 values of omalizumab binding to WT recombinant IgE or mutated IgE (n = 6 per group). G) show the response of omalizumab to recombinant, and H) shows the response of omalizumab to mutated IgE in BLI. D) Shown is the ability of IgE (WT or mutated), previously complexed with omalizumab, to bind to B cells (n = 3 per group). 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 were performed using ordinary Two-way ANOVA with Sidák's multiple comparison test (a = 0.05): ns = not significant, p≤0.05 (*), p≤0.01 (**), p≤0.001 (***), and p≤0.0001 (****)



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**) and **D**) shows the scatter plot of the OD450 value ($n \ge 21$). Shown is the correlation of atopic individuals to anti-mannose lectin and omalizumab. C) or anti-mannose lectin and Le27 D). The color of the dots represents the amount of total IgE: green >100 kU/ml, orange 20-100kU/ml, red <20kU/ml. B and 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%.