Sialylation of IgG antibodies inhibits IgG-mediated allergic reactions

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To the Editor

IgE antibodies (Abs) can mediate allergic reactions, including systemic anaphylaxis by activating the high affinity FcεRI on mast cells and basophils, leading to release of inflammatory mediators[1,2,E1–3] (see References E1–45 in this article's Online Repository). In contrast, allergen-specific IgG Abs, which are also induced by allergen-specific immunotherapies (AIT), can inhibit IgE-mediated anaphylaxis caused by low levels of allergen through allergen-masking and crosslinking of the FcεRI with the classical IgG inhibitory receptor FcγRIIb[2,3,E1,4,5]. However, when allergen levels are high, IgG Abs induced in untreated and SIT-treated allergic patients as well as to medical drugs also have the potential to mediate anaphylaxis by activating classical activating FcγRs on different immune cell types[2,3,4,E6–12].

The effector functions of IgG Abs depend on their subclass[E11,13] and the type of Fc N-glycosylation (Fig 1, A, and Fig E1). Agalactosylated IgG Abs generally promote inflammation[E14,15], whereas galactosylation and terminal sialylation of IgG Abs generally suppress inflammation[5–8,E15–19]. However, the effects of IgG subclass and Fc glycosylation pattern in allergy remain unclear.

Here, we first compared the capacity of differently glycosylated forms of murine IgG1, a subclass that resembles AIT-induced human IgG4 in its limited ability to activate complement and classical activating FcγRs[3,E1,5,11,13], to inhibit IgE-mediated systemic anaphylaxis (Fig 1, B–E, and Fig E1 (see Figs E1–4 and Methods in this article's Online Repository)). IgE-mediated anaphylaxis (assessed as decreased rectal temperature) was induced intravenously (i.v.) with 10 μg of IgE anti-2,4,6-trinitrophenyl (TNP) monoclonal Ab (mAb), followed by an i.v. challenge 24 h later with 1 μg of TNP-coupled ovalbumin (TNP-OVA) (Fig 1, B). Increasing doses of differently glycosylated murine IgG1 anti-TNP mAbs (clone H5; native=low-galactosylated (low-gal), *in vitro* galactosylated (gal) or *in vitro* galactosylated plus sialylated (sialylated; sial)) decreased IgE-mediated hypothermia in a FcγRIIb-dependent manner (Fig 1, B–E, and Fig E1).

Even though low-galactosylated IgG1 showed a tendency for more efficient inhibition (Fig 1, C; 3 μg of IgG1; not significant), possibly due to its higher affinity than sialylated IgG1

for FcγRIIb[16], the IgG glycosylation pattern (Fig 1, *D* and *E*) and IgG subclass (studied by comparing IgG1, IgG2a and IgG2b anti-TNP class switch variant (sv) mAbs with identical V(D)J sequences)[E20] (Fig E1, *G* and data not shown) had only a slight effect on extent of inhibition.

In contrast, the severity of IgG-mediated systemic anaphylaxis, which required challenge with a higher antigen dose (20 μg)[2,3], was IgG subclass- and glycosylation-dependent (Fig 1, *G* and *H*, and Fig E1). De-sialylated plus de-galactosylated (de-gal) IgG2a and IgG2b subclass anti-TNP (sv) mAbs induced more severe anaphylaxis than de-galactosylated (sv) and low-galactosylated (H5) IgG1 mAbs (IgG2a=IgG2b>IgG1) (Fig E1)[4].

IgG1-mediated anaphylaxis was inhibited by galactosylation and especially by additional sialylation (Fig 1, *G*); sialylation also significantly reduced the anaphylaxis potential of IgG2b and tended to reduce that of IgG2a (Fig 1, *G*). Sialylation even reduced the increased anaphylaxis potential of IgG1 in FcγRIIb-deficient mice[4] (Fig 1, *H*), suggesting the importance of additional/other inhibitory mechanisms of IgG1 sialylation, such as one dependent on the C-type lectin receptor, SignR1 (Fig 1, *I*)[7,8,E17,18].

These observations suggest that AIT protocols that promote sialylation of human IgG4 might optimally limit the possibility of IgG-mediated systemic anaphylaxis in the presence of higher allergen doses.

To evaluate this assumption, we analyzed how conventional AIT with birch pollen extract and the adjuvant aluminium hydroxide (alum) (ALK-depot SQ from ALK-Abelló) affects the IgG subclass and glycosylation of anti-Bet v 1 (*Betula verrucosa* 1; the major birch pollen allergen) Abs (Fig E4)[9,E21–23].

In untreated patients, Bet v 1-specific IgG4 titers were constantly low, while IgE but also IgG1 titers increased uring the pollen season (Fig 2, *A*, and Fig E2). In contrast, during AIT, levels of Bet v 1-specific IgG1 increased in the first 12 months but decreased afterwards, while Bet v 1-specific IgG4 titers persistantly increased (Fig 2, *A*, and Fig E2)[1,9,E21,22,24].

However, the Fc glycosylation profile of Bet v 1-specific serum IgG Abs from untreated and AIT-treated patients remained stable and were more highly galactosylated and sialylated than IgG autoAbs from rheumatoid arthritis (RA) patients[E14] (Fig 2, *B* and *C*, and Fig E2). The glycosylation profiles of the AIT-treated patients resembled those of two recently described AIT patients who had received similar therapy with alum (Allergovit from Allergopharma)[5] and of those in therapeutic IVIg, which have Fc sialylation-dependent anti-inflammatory properties (Fig 2, *B* and *C*, and Fig E2)[E16–18].

Consistent with an inverse relationship between IgG sialylation and inflammatory potential, we found that de-sialylation of native Bet v 1-specific IgG from the serum of AIT-treated patients strongly increased its ability to activate neutrophils *in vitro* (Fig 2, *B–E*, and Fig E2).

These observations suggest that conventional AIT with alum induces sialylated IgG(4) Abs that probably have low potential to induce IgG-mediated allergic reactions.

However, studies remain required to assess how Fc glycosylation modulates the effector functions of human IgG1 and IgG4 and how new AIT protocols with distinct adjuvants[1,E25–29], will influence the human IgG subclass distribution and Fc glycosylation pattern and consequently, the risk of IgG-mediated allergic reactions.

To initiate such studies, we compared the effects of enriched complete Freund's adjuvant (eCFA; highly inflammatory), alum and Monophosphoryl Lipid A (MPLA; recently approved for AIT)[1,6,E26,27] on IgG subclass and Fc glycosylation profiles in OVA-immunized mice (Fig 2, *F*, and Fig E3). eCFA induced the highest IgG titer (eCFA>MPLA=alum) (Fig E3, *C*), but all three immunizations induced predominantly IgG1 (alum/94%>eCFA/81%>MPLA/65%) followed by IgG2b and hardly IgG2c (IgG2 (IgG2b +IgG2c): MPLA/35%>eCFA/19%>alum/6%) (Fig 2, *G*, and Fig. E3, *C*), whose functions depend on galactosylation (only IgG1) and sialylation (IgG1 and at least in part IgG2b) (Fig 1, *G*).

In contrast to only small differences in Fc glycosylation pattern between human IgG subclasses in the same sample[30,31], glyopeptide analysis confirmed that murine IgG2 (IgG2b and IgG2c) was, on average, much more highly galactosylated and sialylated than IgG1 (Fig 2, *H*, and Fig E3)[32]. Because alum and MPLA induced higher galactosylation and sialylation levels of both OVA-specific IgG1 and IgG2(b) than OVA-eCFA (Fig 2, *H*), MPLA, with the highest ratio of IgG2(b), induced the highest levels of total IgG galactosylation and sialylation as determined by HPLC glycan analysis (Fig 2, *I*, and Fig E3). Consistent, only 100 µg of purified OVA-specific IgG Abs from the OVA-eCFA group, but not from the OVA-MPLA group, induced IgG-mediated anaphylaxis (Fig 2, *J*).

Taken together, our data suggest that although IgG subclass and glycosylation pattern have relatively little effect on IgG Ab blocking of IgE-mediated anaphylaxis, increased sialylation of IgG(4) Abs should decrease the risk of IgG-induced anaphylaxis in the presence of high allergen doses. Accordingly, it seems advisable to select adjuvants for new AIT protocols[1,E25,26] for their ability to promote sialylated IgG(4) Ab responses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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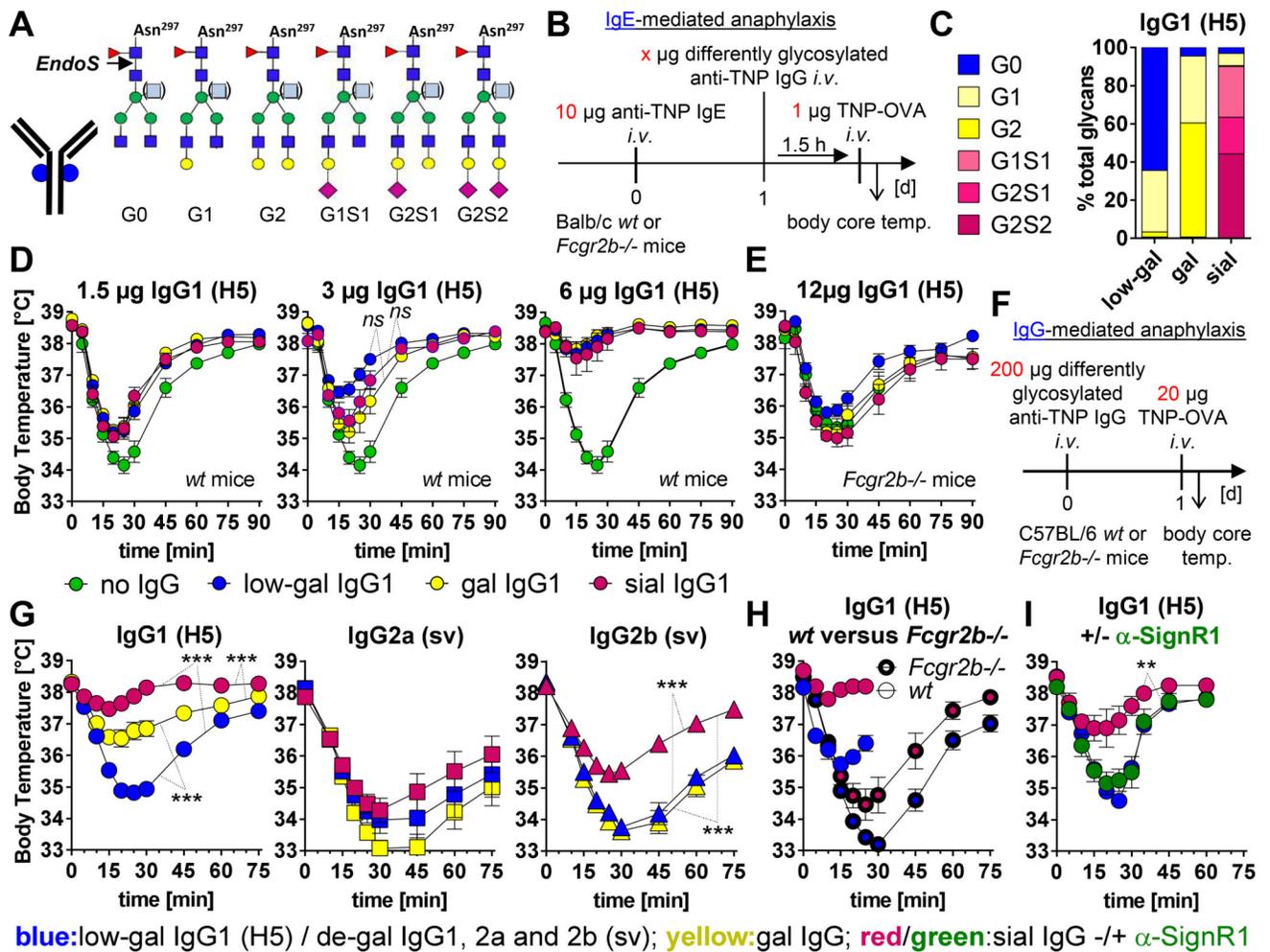
FIG 1. Effect of differently glycosylated IgG subclass Abs on IgE- and IgG-mediated murine anaphylaxis

A, The conserved biantennary N-glycan (four N-acetylglucosamines (dark blue) and three mannoses (green)) at Asn 297 in the IgG Fc part can be modified by fucose (red), bisecting GlcNAc (light blue), galactose (G; yellow) and sialic acid (S; magenta). The cleavage site of *EndoS* used for IgG glycan analysis is depicted. **B,** Experimental design of IgE-mediated anaphylaxis as done in **D** and **E. C,** Fc glycosylation profiles of the differently glycosylated murine IgG1 anti-TNP mAbs (clone H5) that were used in the murine experiments; native=low-galactosylated (low-gal), *in vitro* galactosylated (gal) or galactosylated plus sialylated (sial). **D and E,** Inhibition of IgE-mediated temperature drop by differently glycosylated IgG1 anti-TNP mAbs (H5) in (**D**) wt and (**E**) FcγRIIb-deficient mice (in each graph: no IgG, n=8–10; low-gal, n=8–10; gal, n=5; sial, n=5); symbols represent means. One of two independent experiments is shown. **F,** Experimental design of IgG-mediated anaphylaxis as done in **G–I. G–I,** Decrease in body temperature induced with differently glycosylated IgG subclass anti-TNP mAbs in (**G–I**) wt or (**H**) FcγRIIb-deficient mice (**G–I**) without or (**I**) with α-SignR1 treatment; de-gal: *in vitro* de-sialylated plus de-galactosylated. (**G**) Pooled data (n=10–15) from independent experiments with n=5/group/experiment or (**H, I**) one of two independent experiments is shown.
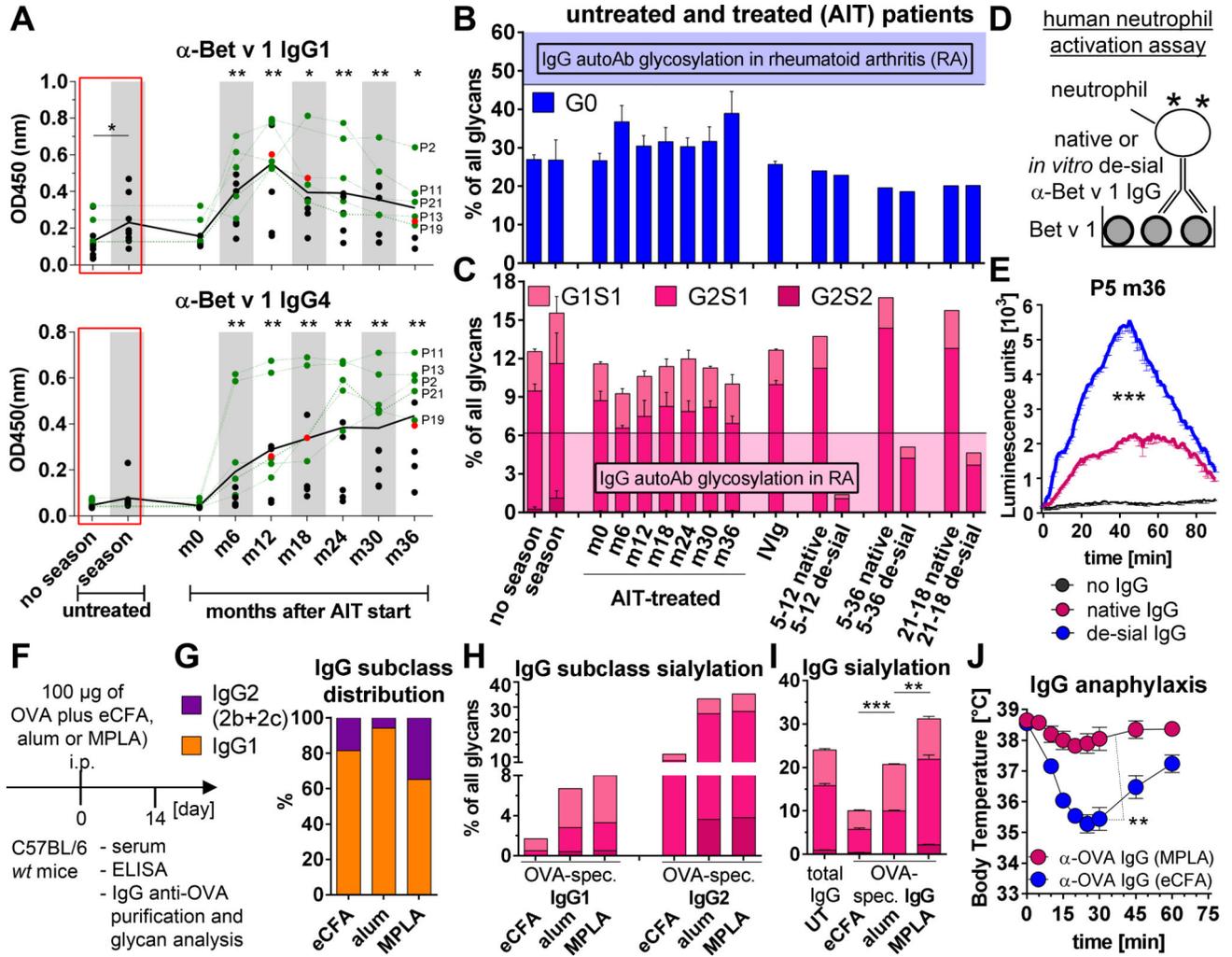
FIG 2. Bet v 1-specific serum IgG Fc glycosylation of untreated and treated allergic patients and influence of different adjuvants on IgG Fc glycosylation

A, Serum titers of Bet v 1-specific IgG1 and IgG4 from untreated (season, n=8; no season, n=6 + 11 (AIT-treated, month (m) 0)) and AIT-treated (n=11) birch pollen allergic patients; black line: mean; gray: pollen season. The green data points depict the 5 AIT-treated patients who were selected for the glycan analysis in **B** and **C** and Fig E2, while the red data points depict the 3 samples (patient 5 at m12 (5–12) and 5–36 and 21-18) that were chosen for *in vitro* de-sialylation and neutrophil activation in **B–E** and Fig E2. One of two independent ELISAs is shown. **B and C**, Percentage of (**B**) agalactosylated (G0) and (**C**) sialylated glycans from purified Bet v 1-specific IgG Abs of untreated (season, n=5; no season, n=5 + 5 (AIT-treated, m0) and the 5 selected AIT-treated patients and from IVIg and purified native and *in vitro* de-sialylated total serum IgG from patient samples 5–12, 5–36 and 21-18. The filled areas indicate the levels of agalactosylated (G0) or sialylated IgG autoAbs in RA patients for comparison[E14]. **D and E**, Human neutrophil activation assay. (**D**) Experimental setup and (**E**) ROS production after activation with native or *in vitro* de-sialylated Bet v 1-specific IgG Abs of patient 5 (P5 m36); no IgG (black). One of two independent ROS assays is shown. **F–J**, The effect of distinct adjuvants (eCFA, alum or MPLA) on the induction of

OVA-specific serum IgG Abs. **F**, Experimental design. **G**, IgG1 and IgG2 (IgG2b+IgG2c; both cannot be distinguished by glycopeptide analysis because of the comparable peptide sequence) frequencies in purified OVA-specific IgG Abs as determined by glycopeptide analysis. **H and I**, (**H**) IgG1 and IgG2 or (**I**) total IgG Fc sialylation profiles of pooled and purified OVA-specific IgG Abs as determined by (**H**) glycopeptide or (**I**) total IgG glycan analysis. **J**, IgG-mediated anaphylaxis as described in Fig 1, *F* with 100 μg of pooled and purified OVA-specific serum IgG Abs; n=4–5 per group. Symbols represent means.