

NEWS AND COMMENTARY

Sialylation regulates activity of IgE in allergic responses

Immunoglobulin E sialylation regulates allergic responses

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Allergic diseases impact hundreds of millions of people worldwide and severe allergic reactions, termed anaphylaxis, can be fatal.¹ Anaphylaxis is most often caused by allergen-specific immunoglobulin (Ig)E antibodies that are bound to the high-affinity Fc epsilon receptor (FcεRI) on mast cells or basophils. Binding of this cell-bound IgE to allergens, in turn, causes cell activation and degranulation which is characterized by the release of inflammatory mediators such as histamine and leukotrienes.² While IgE titer and affinity impact the degree of allergic responses,³ these factors alone do not predict susceptibility to allergy or anaphylaxis. There is a critical need to identify determinants of susceptibility to allergies and to clarify pathways that can be targeted for new treatments.

A recent study by Shade *et al.*, published in the journal *Nature*, makes a substantial contribution to our understanding of allergic predisposition.⁴ Shade *et al.* demonstrate that people with peanut allergies produce IgE antibodies that are enriched for sialic

acid-containing glycoforms. Further, they show that the sialylated glycan structures are critical for IgE-mediated allergic reactions *in vivo* (Figure 1). Finally, they are able to harness this discovery, demonstrating a novel therapeutic approach for allergic diseases. Overall, the study by Shade *et al.* identifies a determinant of susceptibility to IgE-mediated allergies and suggests new diagnostic and therapeutic strategies.

IgE antibodies have seven sites for modification by N-linked glycans and are the most heavily glycosylated of all antibody isotypes.⁵ This suggests a critical role for glycosylation in IgE biology and studies have borne this out. At least one glycan, present at N394 of IgE, is critical for binding to FcεRI.^{6,7} In their previous work, Shade *et al.* showed that the N394 glycan is required for IgE-mediated allergy *in vivo* using a mouse model of passive cutaneous anaphylaxis.⁷

In the present study, Shade *et al.* address the questions of whether IgE glycan composition varies between people who do or do not have allergies, and whether IgE glycan composition impacts its function. They begin their studies by performing a mast cell activation assay in which human mast cells (LAD2 cells) were incubated, or sensitized, with equal amounts of IgEs from peanut-allergic patients or from

nonallergic individuals. The IgE was then cross-linked with a secondary antibody. Interestingly, significantly more degranulation was triggered in the cells that were sensitized with IgEs from peanut-allergic patients. This result suggested intrinsic differences in the IgEs from allergic and nonallergic individuals. Shade *et al.* went on to study the composition of N-linked glycans on IgEs and found that peanut-allergic patients produced IgEs with fewer bisecting N-acetylglucosamine and terminal galactose residues but their IgEs were enriched for sialic acid-containing glycoforms. These experiments revealed functional differences in the IgEs produced by allergic and nonallergic individuals and showed that IgE glycosylation patterns were distinct in people with peanut allergies.

Sialylation of Fc domains is implicated in a variety of activities mediated by IgG, IgA and IgM antibodies.^{8–12} The authors therefore focused on the increased sialoglycan composition of IgEs in allergic patients, asking whether sialylation modulates IgE activity. In a series of experiments in models of passive cutaneous or passive systemic anaphylaxis, Shade *et al.* showed that sialylated IgEs sensitized mice to anaphylaxis while asialylated IgEs did not. IgE sialylation did not impact allergen binding,

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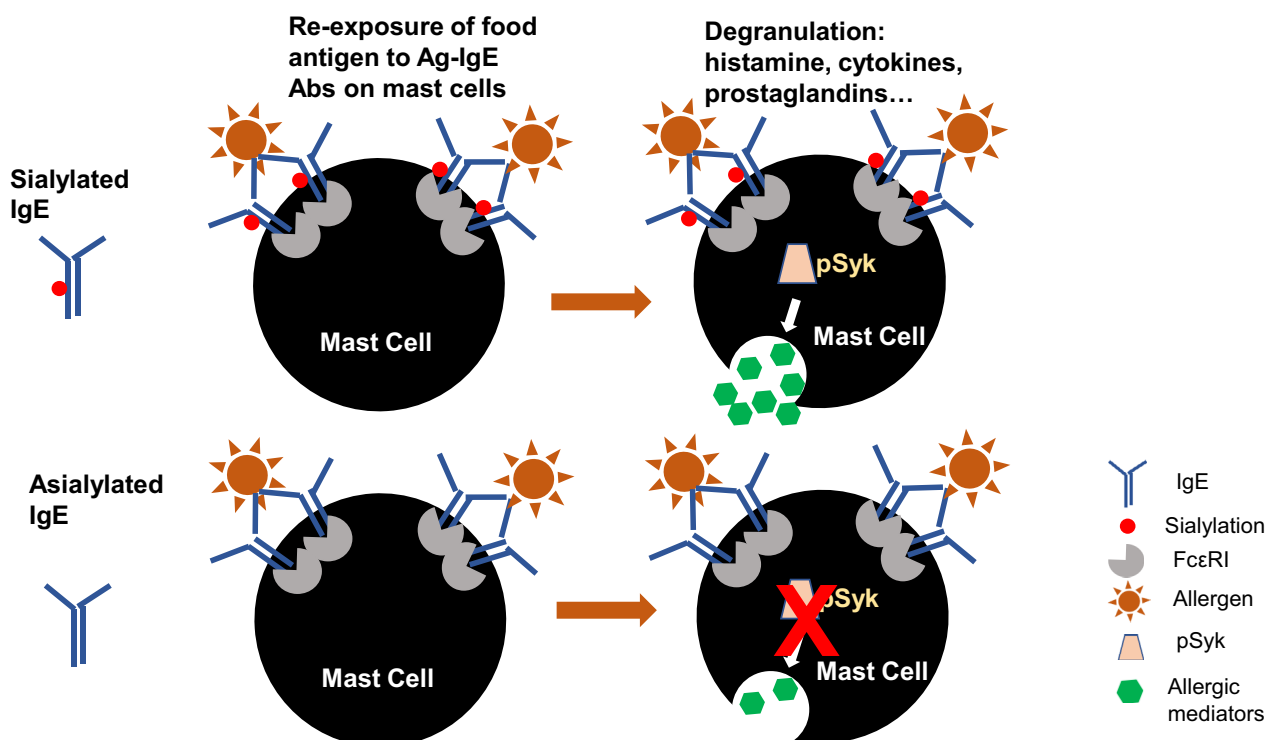


Figure 1. Sialylation on immunoglobulin (Ig)E determines allergy outcomes. Schematic depicting findings in Shade *et al.* Allergic IgE, which is enriched for Fc sialylation, binds to Fc epsilon receptor (FcεRI) on mast cells or basophils and subsequently activates signaling cascades including increased phosphorylation of Syk. This process triggers the degranulation of mast cells to release allergic mediators such as histamine and leukotrienes that contribute to severe allergic symptoms. Abs, antibodies; Ag, antigen; pSyk, phosphorylated Syk.

loading of IgEs onto mast cells and did not significantly change IgE half-life. Shade *et al.* then generated antigen-specific human IgEs that were sialylated or not, and used them to sensitize LAD2 cells or primary human mast cells or basophils. Interestingly, cells sensitized with sialylated human IgEs showed significantly increased degranulation following antigen challenge compared with cells that were sensitized with asialylated human IgEs. These experiments demonstrated that sialylation is a mechanism that regulates the allergic activity of IgE.

Next, Shade *et al.* observed that sialylation of IgE impacted FcεRI signaling; crosslinking of sialylated, cell-bound IgEs resulted in enhanced Syk phosphorylation and calcium flux compared with asialylated IgE. Surprisingly, addition of an excess of asialylated IgE or a non-IgE glycoprotein attenuated activation of

mast cells. This suggested a therapeutic strategy aimed at reducing sialylation of cell surface glycoproteins. To achieve this, the authors engineered a molecule consisting of a sialidase fused to the Fc epsilon domains 2–4 (NEU^{Fcε}). This fusion protein was designed to target FcεRI-bearing cells for removal of sialic acids. Remarkably, incubation of mast cells with NEU^{Fcε} significantly blunted degranulation that was triggered by crosslinking of cell-bound sialylated IgEs. *In vivo*, administration of NEU^{Fcε} significantly attenuated symptoms of IgE-mediated anaphylaxis. This work was a proof of principle for an entirely new therapeutic strategy aimed at modulating sialylation of cells that mediate allergic responses.

Further studies are now needed to clarify the mechanism(s) at play. Of note, previous studies have implicated various glycan-binding

proteins as potential modulators of IgE activity and FcεRI signaling. For example, Niki *et al.* found that galectin-9, which can be secreted by mast cells, interacts with IgE glycans and interferes with antigen binding.¹³ Notably, galectin-9 binds to *N*-acetylglucosamine (LacNAc) structures that would be revealed by sialidase treatment of IgE glycans. It is possible that binding of NEU^{Fcε} to mast cell FcεRI leads to a local increase of terminal LacNAc epitopes on the mast cell surface, resulting in galectin-9 recruitment. In addition, desialylation has been shown to increase internalization kinetics of many cell surface receptors, an effect that could also impact FcεRI signaling.¹⁴

It is interesting that sialylation of IgE seems to promote immune reactivity, whereas this sugar modification in other settings is more

often immune inhibitory. On IgGs, for example, sialylation of the conserved CH2 N-glycans reduces antibody-dependent cell cytotoxicity and promotes anti-inflammatory activity.^{12,15} Furthermore, signaling by sialylated immune complexes on B cells triggers an upregulation of the inhibitory molecule FcγRIIb, elevating the threshold of activating signaling through the B-cell receptor that is required for cell activation.¹¹ Another axis of sialoside-mediated immune modulation involves the sialic acid-binding Ig-type lectin (Siglec) receptor family, many of which downregulate immune cell activity via immunoreceptor tyrosine-based inhibitory motif signaling. Indeed, Duan *et al.* demonstrated that recruitment of mast cell Siglec-3 (CD33) to IgE–FcεRI complexes on the same cells inhibited FcεRI signaling and abrogated an allergic response in mice.¹⁶ Finally, a remaining question is how the glycosylation of IgE is regulated and whether this can be targeted at the level of the B cell or in the serum to reduce the predisposition to allergic diseases.

Overall, Shade *et al.* provided compelling evidence that sialylation of IgE critically regulates its activity. The demonstration that removal of sialic acids on FcεRI-expressing cells can blunt symptoms of anaphylaxis is an extremely exciting development and may guide the development of a new class of treatments for allergic diseases.

AUTHOR CONTRIBUTION

Markus M Xie: Conceptualization and writing. **Carolyn R Bertozzi:**

Conceptualization and writing. **Taia T Wang:** Conceptualization and writing.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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