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Inactivation of IgE and Suppression of Anaphylaxis by Extracts of *Vibrio cholerae*

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Abstract

IgE is known to play a key role in allergy. Mast cells bind IgE via the Fc receptor, FcεRI, and secrete inflammatory mediators via the recognition of allergens bound with IgE. Therefore, IgE is a major target for therapeutic treatment. Previous reports have demonstrated that oligomannose on IgE could be a new target to inhibit allergen function. However, the specific enzyme that modulates IgE for allergy treatment is not yet known. In this study, we found that commercial receptor-destroying enzyme (RDE) from *Vibrio cholerae* culture fluid can specifically modulate IgE, not IgG, and inactivate the initiation of anaphylaxis. RDE-treated IgE was unable to find the binding site of bone marrow derived-mast cells (BMMCs), followed by a reduction in the release of histamine and cytokines. We also confirmed that RDE-treated IgE could not induce passive cutaneous anaphylaxis (PCA) in mouse ears. From these results, we consider that RDE modulates the structure of IgE, rendering it unable to cause allergy. To determine the function of RDE, we focused on the relationship between the modulation and glycosylation of IgE using lectin microarray analysis. We found that RDE-treated IgE significantly reduced the binding to *Lycopersicon esculentum lectin* (LEL) and *Phaseolus vulgaris* leucoagglutinin (PHA-L). These results suggest that RDE specifically modulates branched glycans on IgE, which is then rendered unable to induce an allergic response. These findings could be used in the development of a new drug to inhibit the function of IgE.

Keywords

IgE
Allergy
Glycans
Vibrio cholerae

1. Introduction

Bacterial components such as lipopolysaccharides (LPS) induce various innate immune responses. Many studies have shown how these bacterial components affect immune responses. However, there are many bacterial components whose functions are still unknown. In a previous study, we reported that influenza can be treated with a novel passive immunization method using antibody-expressing genes instead of protein-antibodies [1]. In this study, we found

incidentally that a commercially available cholera extract, receptor-depleting enzyme (RDE), eliminates the ability of neutralizing IgE antibodies to bind to viral antigens. IgE is one of the important factors in the induction of bronchial asthma and anaphylaxis. In recent years, the inactivation of IgE by antibody drugs has been reported to be effective in the treatment of these diseases, but it takes a long time for the effect to appear. Therefore, it is important to develop new treatment modalities targeting IgE. In this paper, we decided to elucidate the mechanism by which RDEs inactivate IgE and investigate whether RDEs could be applied to the treatment of allergy. Our results suggest that IgE-bound sugar chains may be the target of RDEs. In this article, we summarize the relationship between the function of IgE and glycans and the possibility of allergy therapy targeting glycans of IgE [2].

2. Receptor-depleting enzyme (RDE), an extract of *Vibrio cholerae*

Commercially available RDEs derived from extracts of *Vibrio cholerae* have long been used to suppress non-specific reactions when measuring neutralizing antibody titers against influenza viruses [3]. Influenza viruses infect cells by binding to sialic acid, a sugar chain expressed by the host. When measuring virus-specific neutralizing antibody titers induced in serum by vaccine administration or other means, serum, and virus are mixed and added to cells. If the antibodies in the serum have the capacity to neutralize, the cells are protected against infection and survive, but if not, the cells are killed by the virus. However, sialic acid in serum inhibits viral infection of cells in a non-specific manner, as presented in **Figure 1**. Therefore, pre-treatment of serum with RDEs with sialidase activity (mixing RDEs and serum, and reacting them overnight at 37°C) suppresses this non-specific reaction and allows accurate neutralizing antibody titers to be measured [4].

To investigate new influenza treatments, we have generated IgG or IgE antibody genes that bind to haemagglutinin (HA), a viral antigen [1]. The structure of both antibody genes is identical in the variable region and differs only in the constant region. The antibody genes were transfected into HEK293T cells, and the supernatants were treated with RDE and mixed with the virus to determine the neutralizing capacity of anti-HA-IgG or anti-HA-IgE secreted into the culture supernatant. Anti-HA-IgG showed neutralizing ability, while unexpectedly anti-HA-IgE did not show any neutralizing ability [1]. We, therefore, hypothesized that IgE was inactivated by RDE treatment and decided to investigate this in more detail.

3. Mechanism of IgE inactivation by RDE

3.1. RDE-treated anti-HA-IgE loses its antigen-binding capacity

Although the variable regions of anti-HA-IgG and anti-HA-IgE were identical, only anti-HA-IgE lost the ability to neutralize when treated with RDE. Therefore, we first analyzed the antigen-binding capacity of RDE-

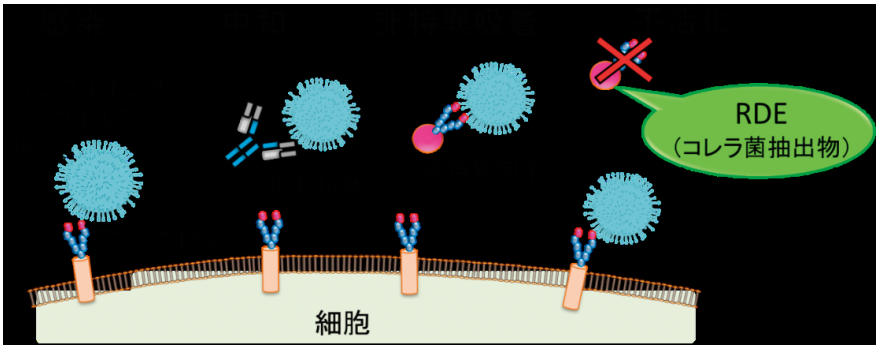

Figure 1. Inactivation of virus non-specific adsorption factors by RDEs. Non-specific factors to which sialic acid is added in serum adsorb non-specifically to the virus and inhibit infection. This makes it impossible to measure the neutralizing capacity of specific antibodies in serum induced by vaccines. Serum can therefore be treated with RDEs to inactivate the non-specific factors.

treated anti-HA-IgG and anti-HA-IgE by ELISA, as shown in **Figure 2A**. According to **Figure 2B**, the antigen binding levels of anti-HA-IgG were almost unchanged with or without RDE treatment. However, the antigen-binding capacity of RDE-treated anti-HA-IgE decreased to the background level. These results suggest that RDE treatment caused anti-HA-IgE to lose its antigen-binding property and consequently no virus neutralizing capacity was observed. Since the variable region and L-chain are common to both HA-IgE and HA-IgE, it has been suggested that RDE may have some effects on the constant region (ε chain) of IgE, thereby reducing its antigen-binding capacity.

3.2. RDE treatment affects IgE structure

To confirm the effects of RDE on the structure of IgE, commercially available purified antibodies were treated with RDE and analyzed by Western blotting. As shown in **Figure 3A** lanes 1–8, no significant change in the major band (approximately 150 kDa) was observed in purified IgG treated or untreated with RDE. On the other hand, the expected size of the untreated IgE band was 190 kDa, while the actual size of the detected band was larger than 250 kDa (**Figure 3A** lanes 9–12). This suggests that IgE may have a special structure due to modifications such as sugar chains. Furthermore, the band size was greatly reduced to about 150 kDa by RDE treatment (**Figure 3A** lanes 13–16). We wondered whether this reduced band contained L-strands or only H-strands. Therefore, purified IgE was treated with RDE under similar conditions and W.B was detected with HRP-labelled L-chain binding protein. In **Figure 3B** lane 2, a band of similar size around 150 kDa was detected in RDE-treated IgE. This result indicates that the band size of RDE-treated IgE is greatly reduced, but the H- and L-strands remain bound. To identify the chain affected by RDE treatment, W.B was performed on RDE-treated IgE under reducing conditions. Firstly, for the L-strand, no significant change in band size was observed with and without RDE treatment for both IgG and IgE (**Figure 3C**). On the other hand, in H-chain, a decrease in the band size of IgE was observed after RDE treatment. Thus, RDEs were found to act on the H chains (ε chains) of IgE, but not on the L chains as predicted above.

3.3. Mechanism of action of RDEs in IgE inactivation: protease? Sialidase? Others?

RDEs are thought to contain several components derived from *Vibrio cholerae* and are known to have protease activity as well as sialidase activity [4]. Firstly, to confirm whether the effect of RDE on IgE was an enzymatic reaction, RDE was heat-treated before acting on IgE. In **Figure 4A**, no reduction in the band

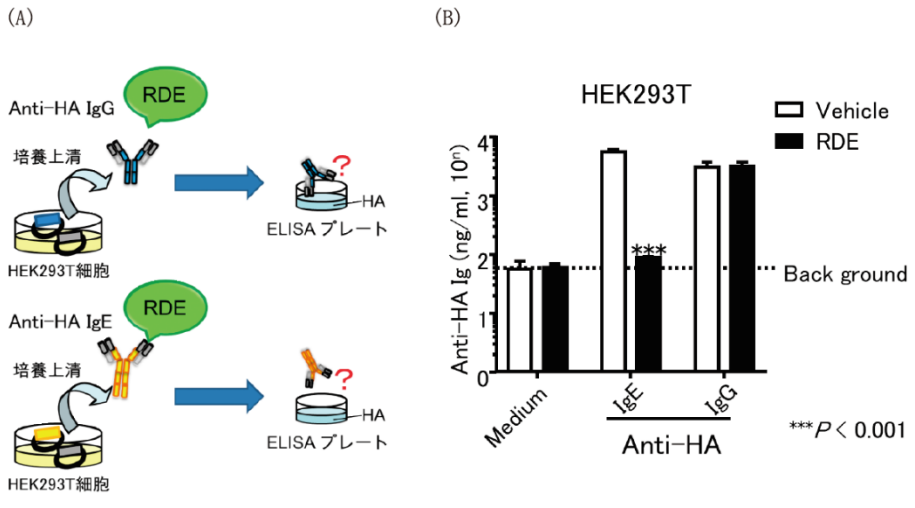

Figure 2. RDE-treated anti-HA-IgE loses antigen-binding capacity [2]. The supernatants of HEK293T cells transfected with anti-HA-IgG and anti-HA-IgE antibody genes were collected and incubated with RDE at 37°C for 6 hours. The antigen-binding capacity of each was then measured by ELISA.

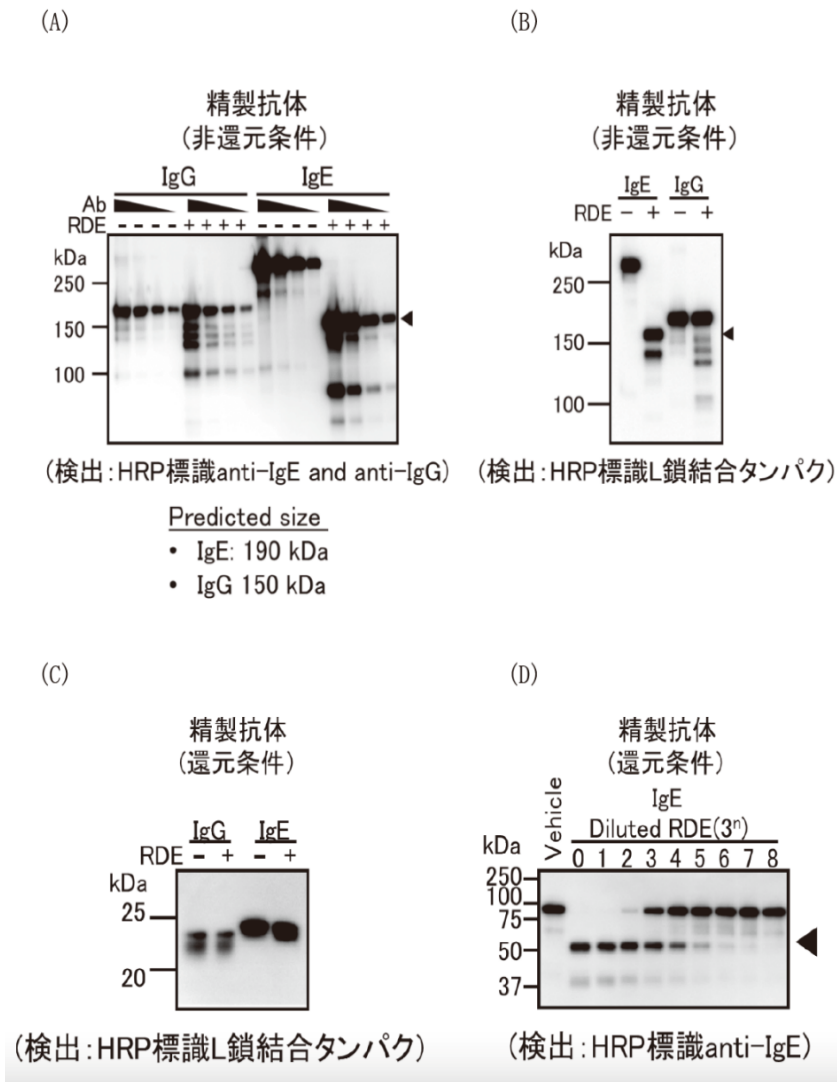

Figure 3. RDEs act on the H-chain of IgE to change its structure [2]. (A-D) Commercially purified IgE or IgG was mixed with RDE and incubated overnight at 37°C. Subsequently, the samples were treated under non-reducing (A, B) and reducing (C, D) conditions, respectively, and analyzed by W.B. For detection of bands, HRP-labelled anti-IgE + anti-IgG (A), HRP-labelled L-chain-binding protein (B, C), and HRP-labelled anti-IgE (D) were used respectively.

size of IgE was observed after heat treatment at 100°C. This suggested that the effect of RDE might be an enzymatic reaction. In another experiment, trypsin was found to reduce IgE band size in the same way as RDEs [2]. Based on this, we hypothesized that proteases might also play a central role in the action of RDEs and studied whether the effect of RDEs on IgE could be blocked by treatment with a protease inhibitor. First, trypsin, used as a control, abolished the effect of the inhibitor on IgE (**Figure 4B** lanes 5 and 6). On the other hand, unexpectedly, the effect of RDE could not be blocked by the protease inhibitor (**Figure 4B** lanes 3 and 4). Another protease inhibitor also failed to block the action of RDE [2], suggesting that the main effect of RDE is not on proteases such as trypsin. Therefore, the sialidase activity was investigated. Specifically, commercially available purified sialidase was used to treat IgE in the same way as RDE. However, it was not found to act in the same way as RDE [2]. This suggests that sialidase is also not the main effect in the IgE inactivation reaction of RDEs. Previous reports have shown that treatment of IgE with PNGase (an enzyme that cleaves most N-linked sugar chains) abolishes its function [5,6]. Therefore, we decided to analyze whether the glycan structure of RDE-treated IgE is also altered.

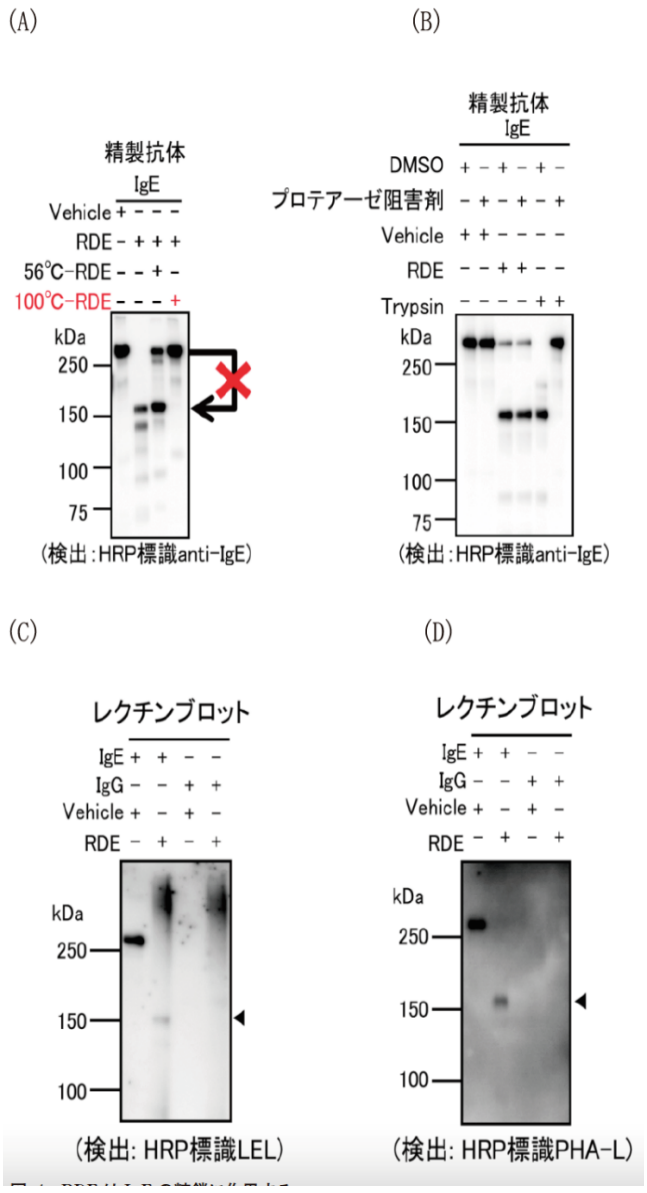

Figure 4. RDEs act on the glycan chains of IgE [2]. (A, B) RDEs were pre-treated with heat treatment (A) or protease inhibitors (B) before being mixed with IgE and incubated overnight at 37°C. Subsequently, analysis was performed in W.B. (C, D) RDE-treated IgE or IgG was analyzed by lectin blotting. HRP-labelled LEL (C) or HRP-labelled PHA-L (D) were used for detection, respectively.

3.4. RDE affects the structure of glycans incorporated into IgE

Lectins, which are glycan-binding proteins, are often used to analyze sugar chains attached to specific proteins. A large number of lectins are used to decipher changes in the structure of the added glycan chains from changes in their binding capacity. A tool for comprehensive analysis of this is the lectin microarray [7]. Using this microarray, we analyzed glycans attached to IgE and altered by the action of RDEs. The results showed that the binding capacity of two lectins, LEL (*Lycopersicon esculentum* lectin) and PHA-L (*Phaseolus vulgaris* leucoagglutinin), was significantly reduced. This was confirmed by lectin blotting, as shown in **Figure 4C** and **4D** lanes 1 and 2. In other words, RDE-treated IgE showed a decrease in band intensity as well as band size when detected with LEL and PHA-L compared to untreated IgE bands. These results suggest that RDE affects the sugar chains attached to IgE. In particular, RDEs affected glycans with binding properties to LEL and PHA-L. Interestingly, LEL and PHA-L did not show binding to IgG, as seen in **Figure 4C** and **4D** lanes 3 and 4. According to previous results, IgG is hardly

affected by the action of RDEs. These results suggest that RDEs may act by specifically recognizing IgE-bound sugar chains.

3.5. IgE functions and glycans

It is known that sugar chains play an important role in the function of antibodies. It is known that antibody-dependent-cellular-cytotoxicity (ADCC) activity increases when fucose is removed from the added sugar chains of IgG [8]. Shade *et al.* reported that among the N-linked carbohydrate chains bound to IgE, the high mannose-type carbohydrate chain bound to domain 3 of the constant domain (Cε3) plays an important role in binding to mast cells [6]. In other words, treatment of IgE with an enzyme that cleaves high mannose-type sugar chains reduced the ability of IgE to bind to mast cells. Wu *et al.* also reported that high mannose-type sugar chains were detected in a similar region in IgE found in the serum of patients with high IgE syndrome [9]. Our study and these previous reports suggest that glycans may also play an important role in IgE function.

4. Potential of IgE-targeted allergy therapy

4.1. Antibody drugs targeting IgE

IgE, known to be one of the main causes of allergy, is induced by allergen exposure, and it binds to FcεRs expressed on the mast cell membrane. Furthermore, when allergens bind to IgE, FcεRs cross-link and cause the release of allergy-causing substances such as histamine. Therefore, allergy treatment targeting IgE is considered to be effective. Antibody drugs against IgE (omalizumab) are already approved for the treatment of bronchial asthma and chronic urticaria. However, omalizumab cannot bind to IgE that is already bound to FcεR, which means that it takes longer to achieve a therapeutic effect [10].

4.2. RDE-inactivated IgE cannot induce anaphylaxis

We, therefore, decided to confirm the ability of RDE-treated IgE to induce anaphylaxis and to investigate whether RDEs could be applied to allergy treatment. For this purpose, we used a model in which anti-dinitrophenol (DNP)-IgE was bound to mast cells, and an allergen (HSA-DNP) was added, causing the release of histamine and other allergy-inducing substances. The results in **Figure 5A** showed that histamine secretion was reduced in RDE-treated IgE. To elucidate the cause, the binding capacity of RDE-treated IgE to mast cells was analyzed by FACS. Based on **Figure 5B**, the results showed that RDE-treated IgE could hardly bind to mast cells. This is thought to be a result of the structural changes in IgE induced by RDE.

To confirm whether there is a similar effect *in vivo*, anti-DNP-IgE was injected into the ears of mice beforehand, and HSA-DNP containing Evans blue dye was injected intravenously. When untreated IgE was administered, anaphylaxis increased vascular permeability and caused Evans blue dye exudation, resulting in a blue area on the ear skin as shown in **Figure 5C**. In contrast, such reactions were rarely observed with RDE-treated IgE. Hence, it was found that RDE-treated IgE had little anaphylaxis-inducing potential *in vivo*.

5. Conclusion

It is thought that the RDEs alter the sugar chains attached to IgE, resulting in a conformational change, which in turn reduces the binding capacity of IgE to mast cells and the secretion of allergy-inducing substances (**Figure 6**). In other words, factors that alter the sugar chain of IgE may be candidates for allergy treatment. It has been known from previous reports that the function of IgE is abolished by cleaving the N-linked sugar chain [5,6]. Furthermore, our study

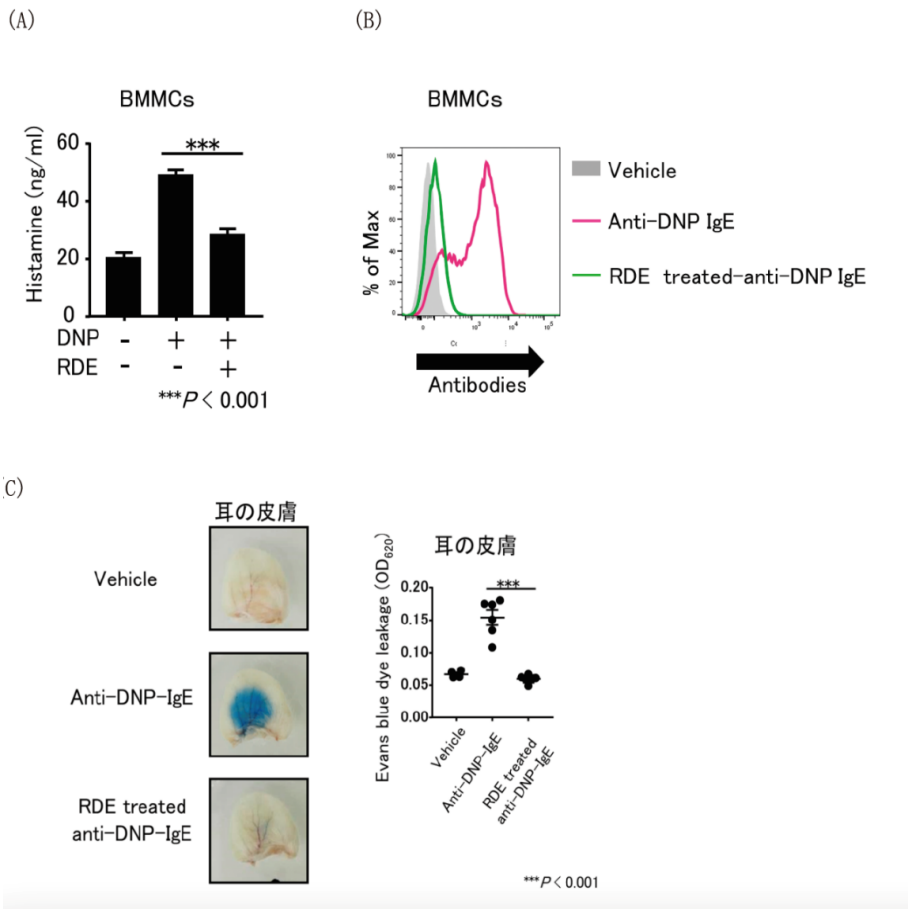

Figure 5. RDE-treated IgE cannot induce anaphylaxis [2]. (A) RDE-treated anti-DNP-IgE was added to mast cells induced from bone marrow, allergen (HSA-DNP) was added and histamine levels in the supernatant were analyzed by ELISA. (B) Binding levels of RDE-treated anti-DNP-IgE to mast cells were analyzed by FACS. (C) RDE-treated anti-DNP-IgE was injected into the ears of mice, followed by intravenous injection of HSA-DNP containing Evans blue dye for analysis of anaphylaxis-induced levels. The right-hand figure shows a graph quantifying the amount of Evans blue dye exudated into the ear skin.

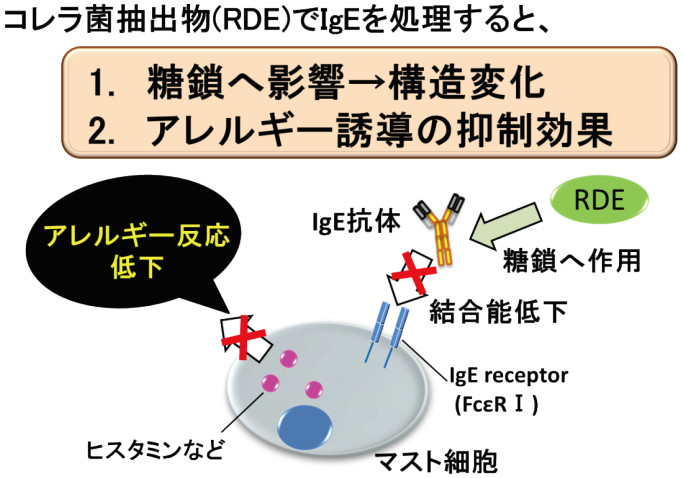

Figure 6. Schematic diagram of IgE inactivation by RDE treatment

suggested that the glycans binding to LEL or PHA-L are important for IgE inactivation. As the action of RDEs was rarely observed in IgG, IgE-specific glycans are likely important for their function. Unfortunately, direct administration of RDEs to mice did not suppress anaphylaxis. However, the identification of factors important for IgE inactivation in the RDEs and further detailed analysis of the target sugar chains could contribute to the development of allergy therapies.

Disclosure statement

The authors declare no conflicts of interest.

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