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Species-specificity in the interaction of IgE with FcεRI between humans and rats

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The interaction of immunoglobulin E (IgE) to its high-affinity receptor (FcεRI) plays a key role in triggering allergic reactions. However, it is still controversial whether species specificity influences the binding ability between humans and rodents. Recombinant hFcεRIα / RBL-2H3 and hFcεRIα / MGC-803 cells were prepared and sensitized with hIgE/anti-hIgE antibody or DNP-IgE/DNP-BSA, respectively. Species-specificity was investigated using immuno-fluorescent analysis, β-hexosaminidase release rate assay, intracellular calcium concentration assay and apoptosis assay, and the results showed that there is species-specificity in IgE/FcεRI in humans and rats, and no cross-recognition between IgE and FcεRI in two species. These results should provide the experimental evidence for further research on the pathogenesis and the drug development of allergic diseases.

1. Introduction

Allergic diseases have reached epidemic proportions in the world today, affecting more than 20 % of the population, but with limited treatment available today (Liu et al. 2011; Mohapatra 2010). The binding of immunoglobulin E (IgE) to its high-affinity receptor (FcεRI) expressed on mast cells and basophiles is essential to the initiation of allergic reactions, which has become a suitable target for anti-allergic drugs (Holgate 2014; Frandsen et al.2013). In recent years, a series of therapeutic allergy drugs have been designed to target the IgE / FcεRI signaling pathway and showed the potential to treat allergic diseases, such as anti-IgE antibodies, anti-FcεRI antibodies, IgE-like polypeptides, receptor analogs, small inhibitory molecules and IgE-based vaccines, etc. (Fung and De 1996).

Previous studies have suggested that there is species specificity in the IgE and FcεRI interacting between horses, dogs and rabbits, but not between humans and rhesus monkeys (Sabban and Ye 2013). However, there is currently no clear conclusion between humans and rodents, which is a major obstacle to allergy-related research, because rodents are the mostly used laboratory animals (Giavina et al. 2014). Most researchers believed that the interaction between humans and rodents is species-specific, human IgE(hIgE) cannot bind to rodents FcεRI (rFcεRI), whereas rodents IgE(rIgE) can easily recognize human FcεRI(hFcεRI) and trigger mast cell degranulation. hFcεRIα can be coupled to endogenous β- and γ- chains of rodents to form a chimeric IgE receptor, which can trigger the activation and degranulation of mast cells (Hakimi et al. 1990; Takagi et al. 2003; Taudou et al. 1993). Some studies have demonstrated that there is no species-specific in humans and rodents, IgE can cross-recognize FcεRI each other and contribute to the activation of allergic reactions (Mohapatra and Qazi 2010; Belostotsky 2004).

Rodents can readily recognize both human and rodents FcεRI, however, it has been questioned whether human IgE can bind only to human FcεRI without binding to rodents. Here, we therefore prepared hFcεRIα/RBL-2H3 and hFcεRIα/MGC-803 cell models to research the common characteristics of allergy reaction, such as: immunofluorescence, β-hexosaminidase release rate, intracellular calcium concentration changes as well as apoptosis. It has been demonstrated that the interaction of IgE and FcεRI has obvious species-specificity between humans and rats.

Abbreviations: AM: Acetyl methyl; DNP-IgE: Monoclonal anti-dinitrophenyl antibody; DNP-BSA: Dinitrophenyl-bovine serum albumin; FACS: Fluorescence activated cell sorting; FcεRI: The high-affinity receptor for the Fc region of immunoglobulin E(IgE); FITC: Fluorescein isothiocyanate; LUVA: Utilizing an immortalized human mast cell line; MGC-803: Metastatic gastric cancer-803; PE: P-phycoerythrin; PBS: Phosphate buffer saline; RBL-2H3: Rat basophile leukemia-2H3

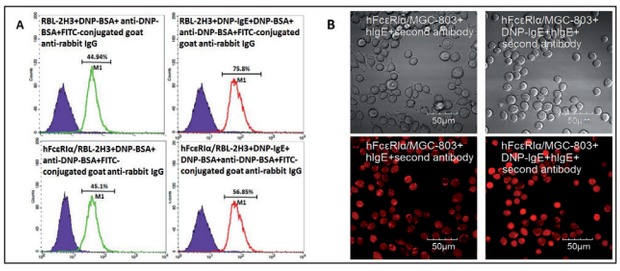

Fig. 1: Binding of rIgE to hFcεRIα. (A) Binding of rIgE to FcεRIα on RBL-2H3cells and hFcεRIα/RBL-2H3 cells by FACS analysis. Blue: Nontreated cells; Green lines: Cells were treated with DNP-BSA for 1h, anti-DNP-BSA for 12 h and FITC conjugated goat anti-rabbit IgG antibody for 1h; Red lines: Cells were incubated with DNP-IgE for 12 h, DNP-BSA for 1h, anti-DNP-BSA for 12 h and FITC conjugated goat anti-rabbit IgG antibody for 1h. M1: population positive for FITC conjugated goat anti-rabbit IgG antibody staining. (B) Competitive binding of rIgE and hIgE to FcεRIα by hFcεRIα/MGC-803 was studied by confocal microscope. Cells (5×10^4 cells/well) were incubated with or without DNP-IgE (0.4 μg/mL for 12 h) following treatment with hIgE (2 μg/mL for 12 h) and PE-labeled mouse anti-hIgE antibody (2 μg/mL for 1 h).

2. Investigations and results

2.1. rIgE binding to RBL-2H3 and hFcεRIα/RBL-2H3 cells

The fluorescence intensity of RBL-2H3 cells and hFcεRIα/RBL-2H3 cells was analyzed by FACS (Fig. 1 A). The fluorescence can be detected both in the positive of RBL-2H3 cells and hFcεRIα / RBL-2H3 cells. Furthermore, the fluorescence intensity of RBL-2H3 cells was significantly higher than that of hFcεRIα / RBL-2H3 cells, both of which were higher than the negative control group. Compared with RBL-2H3 cells, lower fluorescence intensity was detected in hFcεRIα/RBL-2H3 cells treated with anti DNP-IgE. We thought about the lower fluorescence intensity was detected in hFcεRIα/ RBL-2H3 cells in positive treatment, which may be due to hFcεRIα and rFcεRIα competitively bind to endogenous β and γ subunits on the RBL-2H3 cells surface, both chimeric FcεRI and wild-type rFcεRI were expressed on hFcεRIα/RBL-2H3 cells. Therefore, it can be considered that rIgE can interact with rFcεRIα and not recognize hFcεRIα.

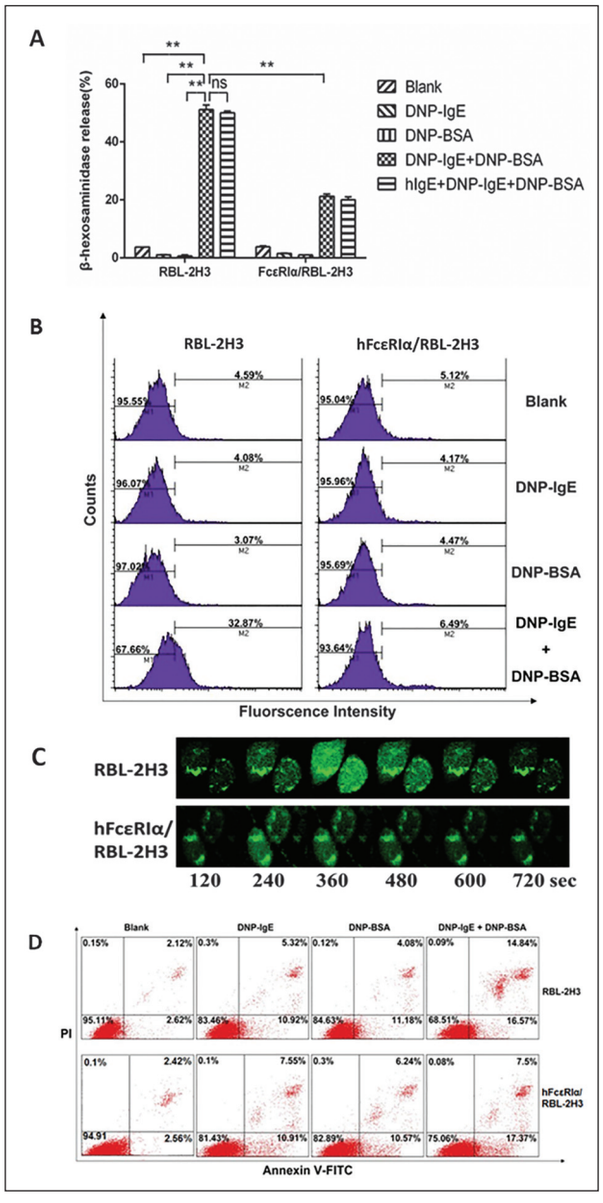

Fig. 2: Activity test of rIgE binding to RBL-2H3 and hFcεRIα/RBL-2H3 cells. (A) Effects of hIgE and rIgE competitive binding to FcεRI on degranulation. All values are presented as mean±SEM for three independent experiments. **p<0.01 vs RBL-2H3 treated with DNP-IgE and DNP-BSA. ns = no significant. (B) FACS analysis the binding of rIgE to FcεRIα on the [Ca^{2+}] mobilization. (C) Olympus confocal microscope analysis rIgE binding to FcεRIα on the [Ca^{2+}] mobilization in cells. (D) FACS analysis the apoptosis on rIgE binding to cells with Annexin V-FITC/PI.

2.2. Activity test of rIgE binding to RBL-2H3 and hFcεRIα/RBL-2H3 cells

2.2.1. β-Hexosaminidase release assay

β-Hexosaminidase has frequently been used as a marker for monitoring degranulation of RBL-2H3 cells. In this study, it was used as an indicator to evaluate the degranulation of RBL-2H3 and hFcεRIα/RBL-2H3 cells induced by DNP-IgE/DNP-BSA (Fig. 2 A). In addition, the β-hexosaminidase release rate in RBL-2H3 cells was significantly higher than that of hFcεRIα/ RBL-2H3 cells. Pretreatment with hIgE before DNP-IgE/ DNP-BSA did not interfere with the change of β-hexosaminidase releasing. Since there was no hFcεRIα subunit on the surface of RBL-2H3, we analyzed that hIgE failed to bind to rFcεRIα subunit to induce degranulation response. Although hIgE binds to the hFcεRIα subunit in hFcεRIα/RBL-2H3 cells, there is no significant change in β-hexosaminidase release rate due to the absence of an agonist.

2.2.2. Intracellular calcium

The biological activity of IgE bound to FcεRIα can be detected by monitoring the intracellular calcium flux changing by FACS

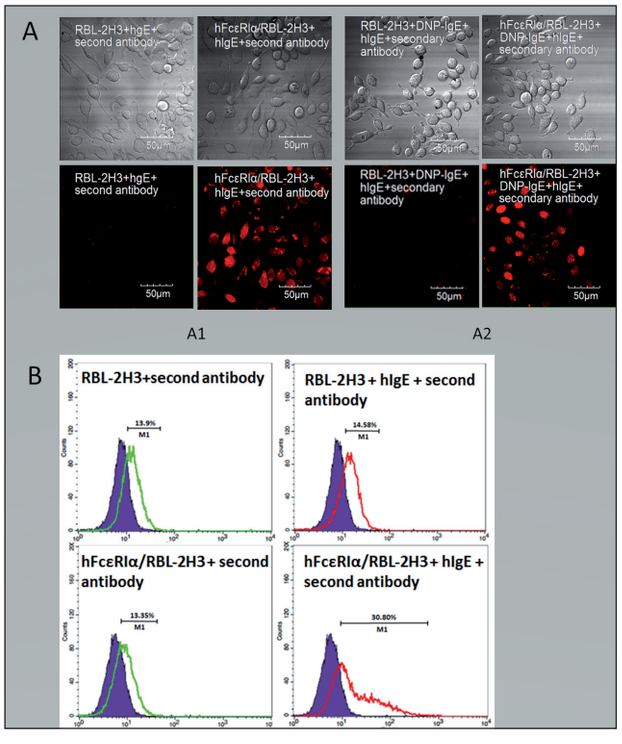

Fig. 3: Binding of hIgE to rFcεRIα. (A) Competitive binding of hIgE and rIgE to FcεRIα by RBL-2H3 and hFcεRIα/RBL-2H3 cells was studied by confocal microscope. (B) FACS analysis binding of hIgE to FcεRIα by RBL-2H3 and hFcεRIα/RBL-2H3 cells exposed to hIgE and PE-labeled mouse anti-hIgE antibody.

(Fig. 2 B). As shown in Fig. 3, DNP-IgE/DNP-BSA can trigger the degranulation of RBL-2H3 cells, and the concentration of cytosolic calcium and the intensity of change were significantly higher than that of hFcεRIα / RBL-2H3 cells. These results show that rIgE specially binds to the rFcεRIα subunit without binding to hFcεRIα to produce the biological activity. The intracellular calcium flux changing from 2 to 12 min was analyzed by confocal microscopy (Fig. 2 C). The changes of calcium currents in RBL-2H3 cells were more significant and strong, while the changes of calcium flux in hFcεRIα/RBL-2H3 cells was weaker. The results are consistent with β-hexosaminidase release assay by FACScan.

2.3. Detection of cell degranulation using the apoptosis method

DNP-IgE/DNP-BSA was used to interact with RBL-2H3 and hFcεRIα/RBL-2H3 cells, respectively. Annexin V-FITC/PI double staining was performed to detect apoptosis (Fig. 2 D). The late apoptosis rate of RBL-2H3 cells was higher than that of hFcεRIα/RBL-2H3 cells. The hypothesis has been that hFcεRIα competes with rFcεRIα for binding to the endogenous β and γ subunits in RBL-2H3 cells to combine the functional hFcεRI and/or rFcεRI, whereas rIgE does not interact with hFcεRIα. Therefore, when DNP-IgE/DNP-BSA was used to interact with hFcεRIα/RBL-2H3 cells, anti-DNP-IgE specially binds with rFcεRIα on the cell surface to activate the degranulation signal and produce low level of degranulation of hFcεRIα/RBL-2H3 in the early stage of apoptosis. Furthermore, there are more rFcεRIα on the surface of RBL-2H3 cells than hFcεRIα/RBL-2H3. Thus, the DNP-IgE binding to the most rFcεRIα can activate degranulation signals and produce a significantly increased degranulation. Therefore, RBL-2H3 cells entered the late stage of apoptosis.

2.4. Binding of rIgE and hIgE to hFcεRIα/MGC-803 cells

MGC-803 cells and hFcεRIα/MGC-803 cells were incubated with DNP-IgE for 12 h and then incubated with hIgE/PE-labeled mouse anti-hIgE. The binding of hIgE and FcεRI was detected by confocal fluorescence microscopy. The results showed that DNP-IgE cannot interfere with the hIgE binding to hFcεRIα to produce the red fluorescence. There was the same fluorescence intensity in the both treatments (Fig.1 B). It can be confirmed that there is no interaction between rIgE and hFcεRI.

2.5. Binding of hIgE to RBL-2H3 cells and hFcεRIα/ RBL-2H3 cells

A clear red fluorescence could be found in hFcεRIα/RBL-2H3 cells (Fig. 3 A1), whereas there was no fluorescence in RBL-2H3 cells after reaction with hIgE/anti-hIgE antibody. The results showed that the hIgE/anti-IgE antibody specifically binds to the hFcεRIα subunit but does not recognize rFcεRIα on RBL-2H3 cells surface. The DNP-IgE and hIgE competitive binding assay showed that there was a similar red fluorescence intensity of hFcεRIα/RBL-2H3 cells between the two treatments, indicating that DNP- IgE cannot bind to hFcεRIα on hFcεRIα/RBL-2H3 cells (Fig. 3 A2), and it cannot prevent the binding of hIgE to hFcεRIα/ RBL-2H3 cells. Meanwhile, there was no fluorescence on RBL-2H3 cells in all treatments.

At the same time, the fluorescent intensity on the cell surface was detected by FACS (Fig. 3 B). On the hFcεRIα/RBL-2H3 cells, the fluorescence of positive cells was significantly higher than that of negative control cells and RBL-2H3 positive cells. The results showed that hIgE/anti-IgE antibody could bind to the hFcεRIα subunit of hFcRIα/RBL-2H3 cells to generate fluorescence signal, while no hFcεRIα subunit on the surface of RBL-2H3 cells, and no fluorescence on RBL-2H3 cells. The results indicated that hIgE does not recognize the rFcεRIα subunit.

2.6. Activity test of hIgE binding to RBL-2H3 and hFcεRIα/RBL-2H3 cells

RBL-2H3 and hFcεRIα/RBL-2H3 cells were incubated with hIgE/PE-labeled mouse anti-hIgE, respectively. The reaction supernatant was used to detect the release rate of β-hexosaminidase. The β-hexosaminidase release in hFcεRIα/RBL-2H3 cells was significantly higher than in the other three groups (Fig. 4). The results demonstrated that hIgE can bind to the hFcεRIα subunit, induce to degranulate and produce β-hexosaminidase, but cannot bind to the rFcεRIα subunit in hFcεRIα/RBL-2H3 cells. While DNP-IgE was added before hIgE, there was no significant difference in the β-hexosaminidase releasing. So we concluded that DNP-IgE cannot interfere with the binding of hFcεRIα and hIgE, and the cells could not be activated. DNP-IgE does not sensitize cells to degranulation without the booster, even though they can bind to rFcεRIα. Both results from the intracellular calcium and apoptotic test were similar with β-hexosaminidase releasing (data not shown).

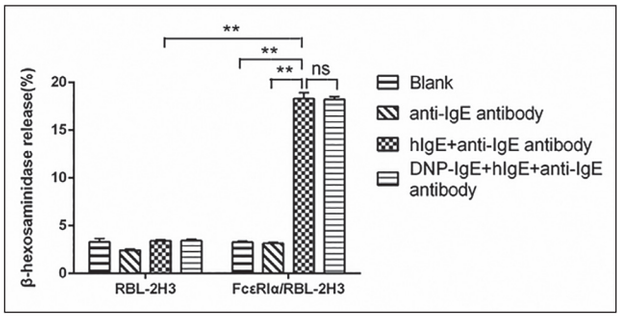

Fig. 4: Effects of hIgE and rIgE competitive binding to FcεRI on degranulation of RBL-2H3 and hFcεRIα/RBL-2H3 cells. All values are presented as mean±SEM for three independent experiments. **p<0.01 vs hFcεRIα/RBL-2H3 treated with hIgE and PE-labeled mouse anti-hIgE antibody. ns = no significant.

3. Discussion

Studies on the IgE/FcεRI signaling pathway has become a hot research topic in the drug development of allergic diseases (Giavina et al. 2014). IgE has two receptors: the high-affinity receptor, FcεRI, and the low-affinity receptor, FcεRII, also known as CD23. FcεRI is suggested to be the key receptor in IgE-mediated allergies. (Nyborg et al. 2016; Zellweger et al. 2017). FcεRI is expressed not only on mast cells (MC) and basophils, but also on human Langerhans cells, peripheral blood dendritic cells (DC), eosinophils, monocytes, polymorphonuclear neutrophils and other cells (Lin et al. 1996). However, FcεRI is expressed only on mast cells and basophils in mice, and eosinophils, monocytes/macrophages, and platelets in rats (Zeng et al. 2016).

FcεRI is expressed in tetrameric form (αβγ2) or trimetric form (αγ2) , whereas αβγ2 is only expressed on mast cells and basophils of human and rodents (Zellweger et al. 2017). The α subunit of extracellular region composed of two immunoglobulin-like domains: D1 domain, associated with high affinity of IgE and FcεRI; D2 domain, a region that binds to the IgE-Fc segment, a transmembrane structure and a cytoplasmic tail (Weetall et al. 1990). The β chains, quadruplex transmembrane antigen receptor, are able to amplify signals and γ chains, linked by disulfide bonds, playing a role in the expression of FcεRI in the cell membrane and intracellular signal transduction (Platzer et al. 2015). Species-specific studies on the role of IgE in binding to FcεRI, have been conducted not only in human and rats but also between human and horses, macaques, and rabbits. The results are not completely consistent, and there are still some arguments (Sabban and Ye 2013). Although rats are widely used as experimental animals, there are still many controversies about the interaction between IgE and their high affinity receptors in humans and rats. The focus of the debate is whether human IgE can only specially bind to human FcεRI but not to the rodent FcεRI (Taudou et al. 1993). Most researchers considered that species specificity exists in IgE-FcεRI interaction between human and rodents (Hakimi et al. 1990; Taudou et al. 1993). The results from transgenic animals have demonstrated hIgE cannot bind to mouse FcεRIα, but the hFcεRIα that can reconstitute a chimeric functional FcεRI with endogenous mouse β and γ chain to trigger mast cell degranulation (Fung et al. 1996). On the other side, a few studies demonstrated that there was no species-specificity in IgE-FcεRI interaction between human and rodents, and human IgE can recognize and sensitize the rat mast cells (Mohapatra and Qazi 2010; Perelmutter 1971). In a recent study, a pro-apoptotic chimeric Fcε-Bak protein was constructed to analyze allergic response. The results revealed that hFcε-Bak can bind to mast cells and basophiles from humans and rodents, indi-

cating that the Fcε fragment of human IgE can bind to the rodent FcεRI (Belostotsky 2004).

The mast cells as typical model were used in the studies for allergic reaction, such as mouse mast cell tumor cells P815, human mast cell HMC-1, LAD2 and LUVA (Kirshenbaum et al. 2014; Sun et al. 2015). With these mast cells as the model *in vitro*, the allergy study is easily disturbed and required the strict operations in experiment, which limits these mast cells be applied *in vitro* (Lee et al. 2015). RBL-2H3 (rat basophile leukemia, RBL), a subfamily of rat basophile tumor cell line, has been wildly used as an early stable and sensitive cell degranulation model for allergy, due to the abundant IgE high-affinity receptors on its membrane and similar characters with mast cells (Rashid et al. 2012). MGC-803 cells, human gastric cancer cells commonly used in recombinant cell research, were selected as negative control cells acting with IgE/FcεRI in this study, because of easy culture, rapid growth *in vitro*, and no FcεRI expression on the cells membranes. The α-chain of human FcεRI(hFcεRIα) was transiently transfected into MGC-803 cells to reveal the interaction between IgE and FcεRI in human and rodents.

As a bridge in the interaction of IgE and FcεRI, the extracellular α subunit is sufficient for IgE binding to FcεRI in the absence of β and/or γ subunit, indicating that the α subunit alone can play a key role in the biological functions of FcεRI with high affinity and specificity to IgE (Sabban and Ye 2013). In this study, the á subunit of human FcεRI was transfected into RBL-2H3 and MGC-803 cells to establish the stable transfected cells compared with the wild RBL-2H3 cells. The results indicated that hIgE could recognize hFcεRIα on the surface of hFcεRIα/RBL-2H3 cells and hFcεRIα/MGC-803 cells, but DNP-IgE could not bind to hFcεRIα and interfere with the binding of hIgE to hFcεRIα on the recombinant cells, indicating that there is no interaction between rIgE and hFcεRI.

The human-mouse chimeric IgE receptors can be expressed onRBL-2H3 cells transfected with hFcεRIα, hIgE can bind to the chimeric FcεRI and have the biological activity (Platzer and Stout 2015). Moreover, the aggregation of hFcεRIα and/or rFcεRIα may lead to a series of signal transduction and biological effects, such as phosphoinositide hydrolysis, calcium ion concentration changing, tyrosine phosphorylation and inflammatory mediator release (Huang et al. 2014).

In this study, the chimeric hFcεRI receptors and wild-type rFcεRI receptors on the surfaces of recombinant hFcεRIα/RBL-2H3 cells could be identified by hIgE and rIgE (DNP-IgE) and produced biological activities, respectively. It may be a defect that the expression level and ratio of chimeric receptor and wild receptor were not detected in the experiment. It is not clear that hIgE or rIgE (DNP-IgE) recognition with its receptors when two receptors are simultaneously expressed on one cell. Though the expression chimeric and wild-type receptors were not examined specifically, nor the effect of co-presence of hIgE or rIgE (DNP-IgE) between the two assays, but it can also ensure the reliability of the results. However, the results are reliable through a variety of treatments on three different cells groups.

Takagi et al. (2003) have observed that intracellular Ca^{2+} concentrations and the β-hexosaminidase releasing were increased in hFcεRIα/RBL-2H3 cells sensitized with hIgE/anti-hIgE antibody or anti DNP-IgE/DNP-BSA, these results were different from our results (Takagi et al 2003). In this paper, the hexosaminidase release rates were significantly higher in RBL-2H3 cell groups than in hFcεRIα/RBL-2H3 cell groups treated by DNP-IgE/DNP-BSA. This result was consistent with the results by the immunofluorescence method. In addition, compared with RBL-2H3 cells, weaker fluorescence was detected on the surface of hFcεRIα/RBL-2H3 cells treated by anti-DNP-IgE. The reason for these results may be that hFcεRIα and rFcεRIα competitively bind to endogenous β and γ subunits to generate chimeric FcεRI or wild-type rFcεRI on the hFcεRIα/RBL-2H3 cells surfaces. Furthermore, the results confirmed that rIgE cannot recognize hFcεRIα through β-hexosaminidase release rate, calcium ion concentration and apoptosis test. We also found that the binding of DNP-IgE to rFcεRI could not be interfered by hIgE as a competitor. Accordingly, when hFcεRIα/RBL-2H3 cells and RBL-2H3 cells were treated by hIgE/PE-anti-hIgE antibody, hIgE could only bind specially to hFcεRIα/RBL-2H3 cells not to RBL-2H3 cells, similar results have been reported in previous studies (Plaut et al. 1973).

In conclusion, through a series of intracellular and extracellular assays detected from recombinant hFcεRIα/MGC-803 cells, hFcεRIα/RBL-2H3 cells and wild RBL-2H3 cells, we have now confirmed there is species-specificity in the interaction with IgE/FcεRI between humans and rats. The results provide the basis for further study on the pathogenesis of allergic diseases. In recent years, it has been found that IgE not only plays a vital role in the allergic reaction but also relates to various diseases such as tumors and auto-reactive diseases, which will attract more attentions to explore the mechanism of IgE/FcεRI (Choi et al. 2016; Josephs et al. 2014; Leoh et al. 2016).

4. Experimental

4.1. Cells and materials

hFcεRIα-pCI-neo/RBL-2H3 and hFcεRIα-pCI-neo/MGC-803 cells were constructed and preserved in our laboratory. Human IgE (hIgE), myeloma was purchased from Calbio chem. Mouse anti-human IgE secondary antibody, PE was purchased from Therom Fisher. Monoclonal anti-dinitrophenyl IgE antibody from mouse was purchased from Sigma. DNP-BSA was purchased from Biosearch Technologies. Anti-BSA was purchased from BBI Life Sciences. FITC-labeled goat anti-rabbit IgG were purchased from BBI Life Sciences. Fluo-3, AM Ester was purchased from Biotium Company.

4.2. Interaction of hIgE and rFcεRI

4.2.1. Binding of hIgE to RBL-2H3 and hFcεRIα/RBL-2H3 cells

pCI-neo-hFcεRIα/RBL-2H3 is a recombinant cell expressing human FcεRI (hFcεRIα). RBL-2H3 cells and hFcεRIα/RBL-2H3 cells were grown on confocal slides (5×10^4 cells/mL) prepared for confocal microscopy. Cells were washed three times with PBS and then incubated with hIgE (0 or 2 μg/mL) for 12 h, followed by incubation with PE-labeled mouse anti-hIgE antibody (2 μg/mL) for 1 h. After repeated washing, the slides were examined and photographed by an Olympus confocal laser scanning system. Meanwhile, cells were collected to analyze by the FACScan. A krypton/argon laser at 565 and 578 nm was used for fluorescence excitation of PE (Spendier 2016).

4.2.2. Activity test of rIgE and hIgE competitive binding to FcεRI

RBL-2H3 cells and hFcεRIα/RBL-2H3 cells were grown in 24-well plates (5×10^4 cells/mL) and preincubated with DNP-IgE (0 or 0.4 μg/mL) for 12 h. Cells were washed twice with PBS and then incubated with hIgE (0 or 2 μg/mL) for 12 h, followed by exposing to PE-labeled mouse anti-hIgE antibody (2 μg/mL) for 1 h. The degranulation was assayed through detecting the release of β-hexosaminidase using an optimized method (Zeng et al. 2016).

4.3. Interaction of rIgE and hFcεRI

4.3.1. Binding of DNP-IgE to hFcεRIα/MGC-803 cells

hFcεRIα/MGC-803 cells were grown on confocal slides (5×10^4cells/mL) prepared for confocal microscopy. Then cells were preincubated with DNP-IgE (0 or 0.4 μg/mL) for 12 h. Cells were washed twice with PBS and then incubated with hIgE (2 μg/mL) for 12 h, followed by exposing to the PE-labeled mouse anti-hIgE antibody (2 μg/mL) for 1 h. After repeated washing, the slides were examined and photographed by an Olympus confocal laser scanning system. A krypton/argon laser at 565 and 578 nm was used for fluorescence excitation of PE.

4.3.2. Binding of DNP-IgE to RBL-2H3 and hFcεRIα/RBL-2H3 cells

RBL-2H3 cells and hFcεRIα/RBL-2H3 cells were grown in 6-well plates 5×10^4 cells/mL) and incubated with DNP-IgE (0 or 0.4 μg/mL) for 12 h. Cells were washed twice with PBS and then stimulated with DNP-BSA (10 μg/mL) for 1 h. The washed cells were incubated with anti-DNP-BSA diluted by 1:500 for 12 h and then stained with FITC labeled goat anti-rabbit IgG diluted by 1:100 for 1 h.The fluorescence intensity was observed by FACScan with a krypton/argon laser at 565 and 578 nm used for fluorescence excitation of PE (Gould and Ramadani 2015).

4.3.3. Activity test of DNP-IgE binding to RBL-2H3 and hFcεRIα/RBL-2H3 cells

RBL-2H3 cells and hFcεRIα /RBL-2H3 cells were grown in 6-well plates (5×10^4 cells/ml) and sensitized with DNP-IgE (0.4 μg/mL) for 12 h. Then, IgE-sensitized cells were washed twice with PBS and incubated with 1 mL Fluo-3 AM for 30 min. The treated cells were washed again with PBS and then stimulated with DNP-BSA (0 or 10 μg/mL). The fluorescent intensity was immediately measured with Olympus confocal laser scanning system using an excitation wavelength of 506 nm and an

emission wavelength of 526 nm. Moreover, cells were analyzed by FACS after treatment with Annexin V-FITC/PI (Lin et al. 1996). Then the release rate of β-hexosaminidase was determinated after 1 h.

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Availability of supporting data: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions: ZCL, YFZ designed the experiments; FL, NNW, YYL,SZ and ZZX performed the experimental work; FL, NNW wrote the manuscript; FL, LFH and CZ analyzed the data and interpreted the results, and all authors read and approved the final manuscript.

Conflicts of interest: The authors declare that they have no competing interests.

References

Belostotsky R, Lorberboum-Galski H (2004) Utilizing Fcepsilon-Bak chimeric protein for studying IgE-FcepsilonRI interactions. Clin Immunol 110: 89 – 99.

Choi YW, Lee KP, Kim JM, Kang S, Park SJ, Lee JM (2016) Petatewalide B, a novel compound from Petasites japonicus with anti-allergic activity. J Ethnopharmacol 178: 17 – 24.

Frandsen PM, Krohn IJ, Hoffmann HJ, Schiotz PO (2013) The Influence of IgE on cultured human mast cells. Allergy Asthma Immunol Res 5: 409 – 414.

Fung-Leung WP, De Sousa-Hitzler J, Ishaque A (1996) Transgenic mice expressing the human high-affinity immunoglobulin (Ig) E receptor α chain respond to human IgE in mast cell degranulation and in allergic reactions. J Exp Med 183: 49 – 56.

Giavina-Bianchi P, Picard M, Caiado J, Mezzano V, Castells MC (2014) LAMP1 and CD63 expression in mouse mast cells and human basophils rendered hyporesponsive by antigen/IgE-mediated activation and desensitization. J Allergy Clin Immun 133: AB59.

Gould HJ, Ramadani F (2015) IgE responses in mouse and man and the persistence of IgE memory. Trends Immunol 36:40 – 48.

Hakimi J, Seals C, Kondas JA, Pettine L, Danho W, Kochan J (1990) The α subunit of the human IgE receptor (FcεRI) is sufficient for high affinity IgE binding. J Biol Chem 265: 22079 – 22081.

Holgate ST (2014) New strategies with anti-IgE in allergic diseases. World Allergy Organ J 7: 17.

Huang J, Zhang T, Han S, Cao J, Chen Q, Wang S (2014) The inhibitory effect of piperine from Fructus piperis extract on the degranulation of RBL-2H3 cells. Fitoterapia 99: 218 – 226.

Josephs DH, Spicer JF, Karagiannis P, Gould HJ, Karagiannis SN (2014) IgE immunotherapy: a novel concept with promise for the treatment of cancer. MAbs 6: 54 – 72.

Kirshenbaum AS, Petrik A, Walsh R, Kirby TL, Vepa S, Wangsa D (2014) A ten-year retrospective analysis of the distribution, use and phenotypic characteristics of the LAD2 human mast cell line. Int Arch Allergy Immunol 164: 265 – 270.

Lee NY, Chung KS, Jin JS, Bang KS, Eom YJ (2015) Effect of chicoric acid on mast cell-mediated allergic inflammation in vitro and in vivo. J Nat Prod 78: 2956 – 2962.

Leoh LS, Daniels-Wells TR, Penichet ML (2015) IgE immunotherapy against cancer. Curr Top Microbiol Immunol 388: 109 – 149.

Lin S, Cicala C, Scharenberg AM, Kinet JP (1996) The FcεRIβ subunit functions as an amplifier of FcεRIγ-mediated cell activation signals. Cell 85: 985–995.

Liu ZC, Shi HL, Zhang YF, Zhao LJ (2011) Progress in the study of allergic disease drugs targeting on IgE/FcεRI signaling pathway. Acta Pharm Sin 46: 1161 – 1166.

Matsuda H, Nakamura S, Yoshikawa M (2016) Degranulation inhibitors from medicinal plants in antigen-stimulated rat basophilic leukemia (RBL-2H3) cells. Chem Pharm Bull 64: 96 – 103.

Mohapatra SS, Qazi M, Hellermann G (2010) Immunotherapy for allergies and asthma: present and future. Curr Opin Pharmacol 10: 276 – 288.

Nyborg AC, Zacco A, Ettinger R, Jack Borrok M, Zhu J, Martin T (2016) Development of an antibody that neutralizes soluble IgE and eliminates IgE expressing B cells. Cell Mol Immunol 13: 391 – 400.

Perelmutter L, Liakopoulou A (1971) Detection of IgE mediated immediate hypersensitivity reactions in the sera of ragweed sensitive individuals using rat mast cells. Int Arch Allergy Immunol 40: 481 – 494.

Platzer B, Stout M, Fiebiger E (2015) Functions of dendritic-cell-bound IgE in allergy. Mol Immunol 68: 116 – 119.

Plaut M, Lichtenstein LM, Bloch KJ (1973) Failure to obtain histamine release from rat mast cells exposed to human allergic serum and specific antigen or IgE myeloma protein and anti-IgE. J Immunol 51: 82 – 83.

Rashid A, Sadroddiny E, Ye HT, Vratimos A, Sabban S, Carey E (2012) Review: Diagnostic and therapeutic applications of rat basophilic leukemia cells. Mol Immunol 52: 224 – 228.

Sabban S, Ye H, Helm B (2013) Development of an in vitro model system for studying the interaction of Equus caballus IgE with its high-affinity receptor FcεRI. Vet Immunol Immunopathol 153: 10 – 16.

Spendier K (2016) N-terminal amphipathic helix of Amphiphysin can change the spatial distribution of immunoglobulin E receptors (FcεRI) in the RBL-2H3 mast cell synapse. Results Immunol 6: 1 – 4.

Sun N, Zhou C, Zhou X, Sun L, Che H (2015) Use of a rat basophil leukemia (RBL) cell-based immunological assay for allergen identification, clinical diagnosis of allergy, and identification of anti-allergy agents for use in immunotherapy. J Immunotoxicol 12: 199 – 205.

Takagi K, Nakamura R, Teshima R, Sawada J (2003)Application of human FcεRIα-Chain-transfected RBL-2H3 cells for estimation of active serum IgE. Biol Pharm Bull 26:252 – 255.

Taudou G, Varin-Blank N, Jouin H, Marchand F, Weyer A, Blank U (1993) Expression of the alpha chain of human FcεRI in transfected rat basophilic leukemia cells: functional activation after sensitization with human mite-specific IgE. Int Arch Allergy Immunol 100: 344 – 350.

Weetall M, Shopes B, Holowka D, Baird B (1990) Mapping the site of interaction between murine IgE and its high affinity receptor with chimeric Ig. J Immunol 145: 3849 – 3854.

Zellweger F, Gasser P, Brigger D, Buschor P, Vogel M, Eggel A (2017) A novel bispecific DARPin targeting FcgammaRIIB and FcepsilonRI-bound IgE inhibits allergic responses. Allergy 72: 1174 – 1183.

Zeng HR, Wang B, Zhao Z, Zhang Q, Liang MY, Yao YQ (2016) Effects of Viola yedoensis Makino anti-itching compound on degranulation and cytokine generation in RBL-2H3 mast cells. J Ethnopharmacol 189: 132 – 138.