



Activation-induced cytidine deaminase plays crucial role in ovalbumin-induced food allergy and promoted by IL-21

Yongning Li^{a,1}, Yongjun Wang^{b,1}, Nanan Sun^a, Hui Yang^a, Qiannan Zhang^a, Xiaopeng Zhang^a, Run Huang^c, Xudong Jia^{a,*}

^a NHC Key Laboratory of Food Safety Risk Assessment (China National Center for Food Safety Risk Assessment), Beijing, China

^b Shandong Provincial Qianfoshan Hospital, the First Hospital Affiliated with Shandong First Medical University, Jinan 250014, China

^c Food Science and Engineering College, Beijing University Of Agriculture, Beijing 102206, China

ARTICLE INFO

Keywords:

Aicda
Food allergy
IL-17A
IL-21
STAT3

ABSTRACT

The prevalence of IgE-mediated food allergy is increasing in the whole wide world which often causes skin and gastrointestinal tract symptoms, or even fatal anaphylactic shock. However, the evaluation of food allergens remains difficult, and the mechanism of food allergy is still not fully clear. To study the gene expression profile in food allergy animal models and identify the regulatory mechanism of the crucial genes, two administration routes were used to build animal models in our study. OVA-specific IgE and IL-4 levels were tested by ELISA, transcriptome profiling was carried out by microarray, and the regulatory mechanism of the highest expressed gene was studied in the primary spleen cells. We found that activation-induced cytidine deaminase (Aicda) is the highest expressed gene in the allergic mice, IL-21 can dramatically enhance the expression of Aicda in the lymph node microenvironment, and IL-17A can promote this effect significantly though it has only limited influence by itself. At last, we illuminated that the promotion of IL-21 on Aicda is partially through STAT3. In summary, our results suggest that IL-21 and IL-17A may play important role in the expression of Aicda as well as food allergy.

1. Introduction

Food allergy is a toxicological effect arising from a specific immune response to a given food. The incidence of food allergy in western countries is probably 1–3% in adults and 3–8% in infants, and may be increasing in prevalence (Boyce et al., 2010; Lack, 2008; Rona et al., 2007). Allergic reactions mediated by food-specific IgE can elicit rapidly (minutes up to 2 h) after the exposure to allergens, patients with food allergy often present skin and gastrointestinal tract symptoms, and a very few can even go into fatal anaphylactic shock (Kulis et al., 2015). Unfortunately, although tremendous progress has been made in recent years, the evaluation of food allergens remains difficult, and the mechanism of food allergy is not yet fully demonstrated, hence, the only method for patients with food allergy to protect themselves is strictly avoidance of all potential allergen-containing food products (Liu et al., 2016). Furthermore, the introduction of new or modified proteins into human diet also pose a challenge to the development of methods for assessing undetermined food allergens. For this reason, it is necessary and essential to develop suitable animal models which could provide valuable research tools for the assessment of food allergy (Bogh et al.,

2016).

Antigen-specific IgE antibody producing is a prerequisite for the development of food allergy. In the sensitization phase of food allergy, B cells are activated by Th2 responses to produce antigen-specific IgE antibody through programmed DNA damage including class switch recombination (CSR) and somatic hypermutation (SHM). In both of these two processes, **Activation-induced cytidine deaminase** (Aicda) has been shown to play a central role (Muramatsu et al., 2000). Therefore, the regulation of Aicda might be critical for the production of food-specific IgE and the development of food allergy.

The induction of Aicda requires engagement of CD40 by CD154 and BCR signal which can be synergized by specific cytokines (Zan and Casali, 2013). Previous studies showed that Aicda can promote the secretion of IgE in human naive B cells by activating STAT3 (Avery et al., 2008; Jérôme Pène et al., 2004; Kobayashi et al., 2009). However, IL-21 turns to inhibit the production of IgE in the mice (Suto et al., 2002). In conclusion, the role of IL-21 in the allergy of human and murine is contradictory and needs to be further studied. Meanwhile, it is reported that IL-17A is also able to induce Aicda and IgE expression in human B cells (Milovanovic et al., 2010). Another study showed that

* Corresponding author at: 7 Panjiayuan Nanli, Beijing 100021, China.

E-mail address: jiaxudong@cfsa.net.cn (X. Jia).

¹ These authors contributed equally.

IL-17 can upregulate *Aicda* in BXD2 mice indirectly by elevating the expression of *Rgs13* (Wang et al., 2013).

In this study, we investigated the mechanism of ovalbumin (OVA)-induced food allergy using two animal models. Through transcriptomic analysis, we identified that *Aicda*, *Rgs13* and *Mybl1* are consistently over-expressed in animals administered with OVA via gavage or intraperitoneal injection. The effect of IL-21 and/or IL-17 on the expression of *Aicda* was studied in primary spleen cells, we found that IL-21 can induce dramatic over-expression of *Aicda* in the whole spleen cells than that in the purified spleen B cells stimulated with IL-4 and anti-mouse CD40 mAb, which might be predominantly dependent on the activation of STAT3. In contrast, IL-17A only has little influence on *Aicda* expression but can cooperate with IL-21 to enhance its promoting effect.

2. Materials and methods

2.1. Reagents

Ovalbumin was purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies for immunohistochemistry against *Aicda*, IL-21, IL-17A and Goat Anti-Rat IgM μ chain (HRP) were purchased from Abcam (Cambridge, MA, USA). Antibody for GL7 was purchased from Thermo Fisher Scientific (San Diego, CA, USA). Recombinant Mouse IL-21, IL-17A, IL-17B and IL-4 were purchased from R&D Systems Inc (Minneapolis, MN, USA). Anti-mouse CD40 mAb was obtained from BD Biosciences (Franklin Lakes, NJ, USA). Niclosamide (STAT3 inhibitor) was purchased from selleckchem (Munich, Germany).

2.2. Animals

C3H/HeJ mice were purchased from Model Animal Research Center of Nanjing University (Nanjing, China). C3H/HeN and BALB/c mice were provided by Vitalriver (Beijing, China). Animals were housed in a controlled environment with a 12 h light/dark cycle, and given water ad libitum. The diet did not contain any allergens that could influence the outputs of the experiments. The study designs were approved by the Institutional Animal Care and Use Committee of China National Center for Food Safety Risk Assessment (CFSA).

2.3. Oral-sensitized animal models

Six-week-old male and female C3H/HeJ or C3H/HeN mice were randomly assigned into two groups. Mice in OVA groups were gavaged with OVA at 40 mg/kg body weight per day for 14 or 28 consecutive days. The control groups were treated with the solvent vehicle (normal saline). At the end of the experiment, animals were anesthetized with pentobarbital and humanely sacrificed after blood sample collection. Blood samples and tissues of spleen and Peyer's Patches were harvested for the analysis of IL-4, OVA-specific IgE and Affymetrix GeneChip Mouse Genome 430 2.0 Array, respectively.

2.4. IL-4 analysis

The spleens of the experimental animals were removed and freshly prepared for single-cell suspensions by mincing through cell strainers. The splenocytes were cultured in RPMI-1640 medium at concentration of 1×10^7 /ml in 6-well plates with 20 μ l sterile OVA solution (0.5 mg/ml) for 2 days, all mediums were supplemented with 10% fetal bovine serum, penicillin (100 μ g/ml) and streptomycin (100 μ g/ml). Then, cell culture supernatants were harvested for the analysis of cytokine IL-4 using the mouse IL-4 high sensitivity ELISA kit (eBioscience, Vienna, Austria).

2.5. OVA-specific IgE analysis

Blood samples were collected from the experimental animals and centrifuged at 2500 r/min for 15 min at 4°C to obtain serums. Serum OVA-specific IgE levels were determined using the mouse anti-OVA IgE antibody assay kit (Chondrex, Inc., Washington, USA) according to the instruction.

2.6. Affymetrix GeneChip mouse genome 430 2.0 array

The Affymetrix GeneChip Mouse Genome 430 2.0 Array (Affymetrix, USA) was performed for transcriptome profiling at Beijing CapitalBio Corporation (Beijing, China). Total RNA was extracted from the snap-frozen Peyer's Patches using Trizol reagent (Invitrogen, Carlsbad, CA, USA). DNase I and RNeasy Kits (Qiagen, Hilden, Germany) were used to eliminate the contaminating DNA and purify the total RNA, respectively. The differentially expressed genes were identified with the Significance Analysis of Microarrays (SAM) system, which could provide a statistically significant changing-fold and a false discovery rate (FDR) of a gene. A threshold value of ≥ 1.5 or ≤ -1.5 -fold change and a FDR significance level of $< 5\%$ were used to select the differentially expressed genes in this experiment. All microarray data are available from the Gene Expression Omnibus (GEO) database using series accession number [GSE117916](#).

2.7. Intraperitoneal injection-sensitized animal model

Six-week-old male and female BALB/c mice were randomly assigned into two groups, separately. Mice in OVA groups were injected intraperitoneally with OVA at 2 mg/kg body weight for 3 times (days 1, 3, and 5). Mice in control groups were treated with the solvent vehicle (normal saline). At the end of the experiment, animals were anesthetized with pentobarbital and humanely sacrificed after blood sample collection at 14th and 28th day, separately. The spleens of the experimental animals were harvested for the Agilent mouse lncRNA + mRNA Array V1.0 and the following PCR analysis.

2.8. Agilent mouse lncRNA + mRNA array V1.0

The Agilent mouse lncRNA + mRNA Array V1.0 (Agilent, USA) was performed at Beijing CapitalBio Corporation (Beijing, China). Total RNA containing small RNA was extracted from the snap-frozen spleens by using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) and purified with mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA). The lncRNA + mRNA array data were analyzed for data summarization, normalization and quality control by using the GeneSpring software V13.0 (Agilent). To select the differentially expressed genes, we used threshold values of ≥ 2 and ≤ -2 -fold change and a *t*-test *P* value of 0.05. All microarray data are available from the Gene Expression Omnibus (GEO) database using series accession number [GSE117916](#).

2.9. QuantiGenePlex assay

To verify the results of microarray, a QuantiGenePlex Assay from Affymetrix based on branched DNA signal amplification technology with xMAP beads was performed at the Gene Company Limited (Beijing, China) according to manufacturer's instruction. All data were normalized against the housekeeping genes.

2.10. Real-time PCR

RT-PCR was carried out using the Bio-Rad IQ5 Multicolor Real-time PCR Detection System. Total RNA was isolated by Beijing CapitalBio Corporation (Beijing, China) as described previously. cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Thermo, Rockford, IL, USA). The real-time PCR Master Mix (SYBR

Green) was purchased from Research Biolab Co., Ltd (Beijing, China).

2.11. Immunohistochemistry

For paraffin embedded tissue section, antigens were unmasked in citrate buffer for 10 min at 95 °C. Endogenous peroxidase was quenched by incubating in 3% hydrogen peroxide for 10 min. Then after blocking with 5% normal goat serum, the sections were incubated with primary antibody overnight at 4 °C. Finally, HRP-conjugated secondary antibody and diaminobenzidine (DAB) were applied to visualize the expression and localization of antigens.

2.12. Primary spleen cells culture

To obtain whole spleen cells or purified spleen B cells, spleens from naive or pre-immunised mice were removed and freshly prepared for single-cell suspensions by mincing through cell strainers. B cells were negatively isolated with Mouse B Lymphocyte Enrichment Set-DM (BD Biosciences, Franklin Lakes, NJ, USA). Purified B cells or whole spleen cells were cultured in complete RPMI-1640 medium containing 10% fetal bovine serum, penicillin (100 µg/ml), streptomycin (100 µg/ml) and seeded at 1×10^7 cells/well in a final volume of 1.5 ml in 6-well plates for 48 h in the presence and absence of OVA (1 µg/ml), anti-mouse CD40 mAb (50 ng/ml), IL-4 (50 ng/ml), IL-17A (50 ng/ml), IL-17B (50 ng/ml) or IL-21 (50 ng/ml), respectively.

2.13. Statistical analysis

The SPSS Statistical Software (SPSS for Windows 18.0, Chicago, USA) was used to analyze the data. Gene expression levels were expressed as mean $\pm$ standard deviation and analyzed using one-way Analysis of Variance (ANOVA). Dunnett's two-tailed *t*-test was used for testing if any treatment group is significantly different from the control group, the *P* value < 0.05 was considered statistically significant.

3. Results

3.1. Affymetrix GeneChip mouse genome 430 2.0 array

To carry out Affymetrix GeneChip Mouse Genome 430 2.0 Array, two strains of C3H mice including C3H/HeJ and C3H/HeN were used to establish oral-sensitized animal models. After the treatment with OVA for 28 days (Fig. 1A), mice which were detected with high levels of both OVA-specific IgE and IL-4 (Fig. 1B) were classified as allergic mice. The sensitization rates of the two strains were basically the same judged by OVA-specific IgE and IL-4 levels, but the consistency of OVA-specific IgE and IL-4 in the C3H/HeJ mice was better than that of the C3H/HeN mice (Supplementary figure S1), so we chose the Peyer's Patches of the C3H/HeJ mice to do the mRNA microarray.

With a predefined threshold of ≥ 1.5 -fold, 807 genes were considered to be differentially expressed, including 596 genes (red dots) up-regulated and 211 genes (green dots) down-regulated. The Kyoto Encyclopedia of genes and genomes (KEGG) pathway database was used to analyze enriched signaling pathway components related to the differentially expressed genes. The result showed that intestinal immune network for IgA production, primary immunodeficiency and B cell receptor signaling pathway were significantly enriched (Fig. 1C).

3.2. Agilent mouse lncRNA + mRNA array V1.0 and QuantiGenePlex assay

To carry out Agilent mouse lncRNA + mRNA Array V1.0, BALB/c mice intraperitoneal injection-sensitized animal model was used. After three times of injections, the experimental animals were executed at the 14th or 28th day, separately (Fig. 2A). The serum concentration of OVA-specific IgE was tested and used as the basis for selecting

experimental animals to perform the microarray (Fig. 2B), and only female mice were used as their reactions to OVA were more sensitive than males as shown in the serum OVA-specific IgE levels.

With a predefined threshold of ≥ 2.0 -fold, 22 genes were differentially expressed at the 14th day (14 genes up-regulated and 8 genes down-regulated) and 397 genes were differentially expressed at the 28th day (388 genes up-regulated and 9 genes down-regulated), among all these genes *Aicda*, *Rgs13* and *Mybl1* were highly expressed in both of the Affymetrix GeneChip Mouse Genome 430 2.0 Array and the Agilent mouse lncRNA + mRNA Array V1.0 (both of the 14th and 28th day, Fig. 2C).

To verify the chosen differentially expressed genes from the two microarray assays, we carried out a QuantiGenePlex Assay with the same mRNA samples (samples from male mice were included) used in the Agilent mouse lncRNA + mRNA Array V1.0 array. The result of the QuantiGenePlex Assay was consistent with microarrays and showed that *Aicda*, *Rgs13* and *Mybl1* were significantly highly expressed in the female mice at both of the 14th and 28th days, and there was an obviously downward trend from the 14th day to the 28th day (Fig. 2D). Although the three genes in the male animals were highly expressed at the 14th day but the levels of these genes of the control group were relative high at the 28th day, which provided further evidence that allergic reaction in the female mice was more specific than in the males.

To further confirm the expressions of *Aicda*, *Rgs13* and *Mybl1* in female mice, RT-PCR was performed. As shown in the Fig. 2E, the results of the RT-PCR was basically consistent with the QuantiGenePlex Assay, except for the *Aicda* showed to be the most highly expressed gene at the early stage of allergy.

3.3. High-level expression of *Aicda* was consistent with the clinical manifestations of allergic mice and co-expressed with IL21 and IL-17A in the Peyer's Patches

Previous studies showed that *Aicda* mediates two central processes in antibody diversification including CSR and SHM, and IgE generation can be influenced by both of the IL-21 and IL-17 (Klein and Dalla-Favera, 2008; Kobayashi et al., 2009; Lajoie et al., 2014; Milovanovic et al., 2010; Zan and Casali, 2013). In this study, we investigated the co-expression of *Aicda*, IL-21 and IL-17A in the Peyer's Patches of the OVA induced allergic mice. Female C3H/HeN mice were gavaged by OVA for 14 days, when administration was finished all the mice were challenged with 1 mg OVA by intraperitoneal injection (Fig. 3A). As shown in Fig. 3B, two obvious clinical symptoms were observed after challenging, including lips swelling and activity reduction. In the Peyer's Patches of the mice which showed clinical signs, *Aicda* was significantly highly expressed compared with the control group and IL-21 elevated with a lower extent, and we also found that *Aicda*, IL-21 and IL-17A are co-expressed in the areas of GL7+ cells (Fig. 3C). Taken together, these data indicated that *Aicda* is strongly expressed in the lymph gland of the allergic mice while IL-21 and IL-17A may be associated with the elevated expression of the *Aicda*.

3.4. The promoting effect of IL-21 and IL-17A on the expression of *Aicda* in the primary spleen B cells is limited

To clarify the relation between IL-21, IL-17A or IL-17B with *Aicda*, primary spleen B cells culture was performed. First, the spleens from naive mice or pre-immunised mice which had underwent three times of injections of OVA were harvested and purified B cells were isolated with magnetic beads (Fig. 4A and B). With supplement of OVA, IL-4 and anti-mouse CD40 mAb in the presence and absence of IL-21, IL-17A and IL-17B, we found that IL-21 could only slightly elevate the expression of *Aicda* in the naive B cells but not in the pre-immunised B cells, meanwhile, IL-17A and IL-17B could reduce *Aicda* levels in both of the two kinds of B cells (Fig. 4C and D). These data suggested that IL-21, IL-17A and IL-17B could not enhance the expression of *Aicda* in the purified B

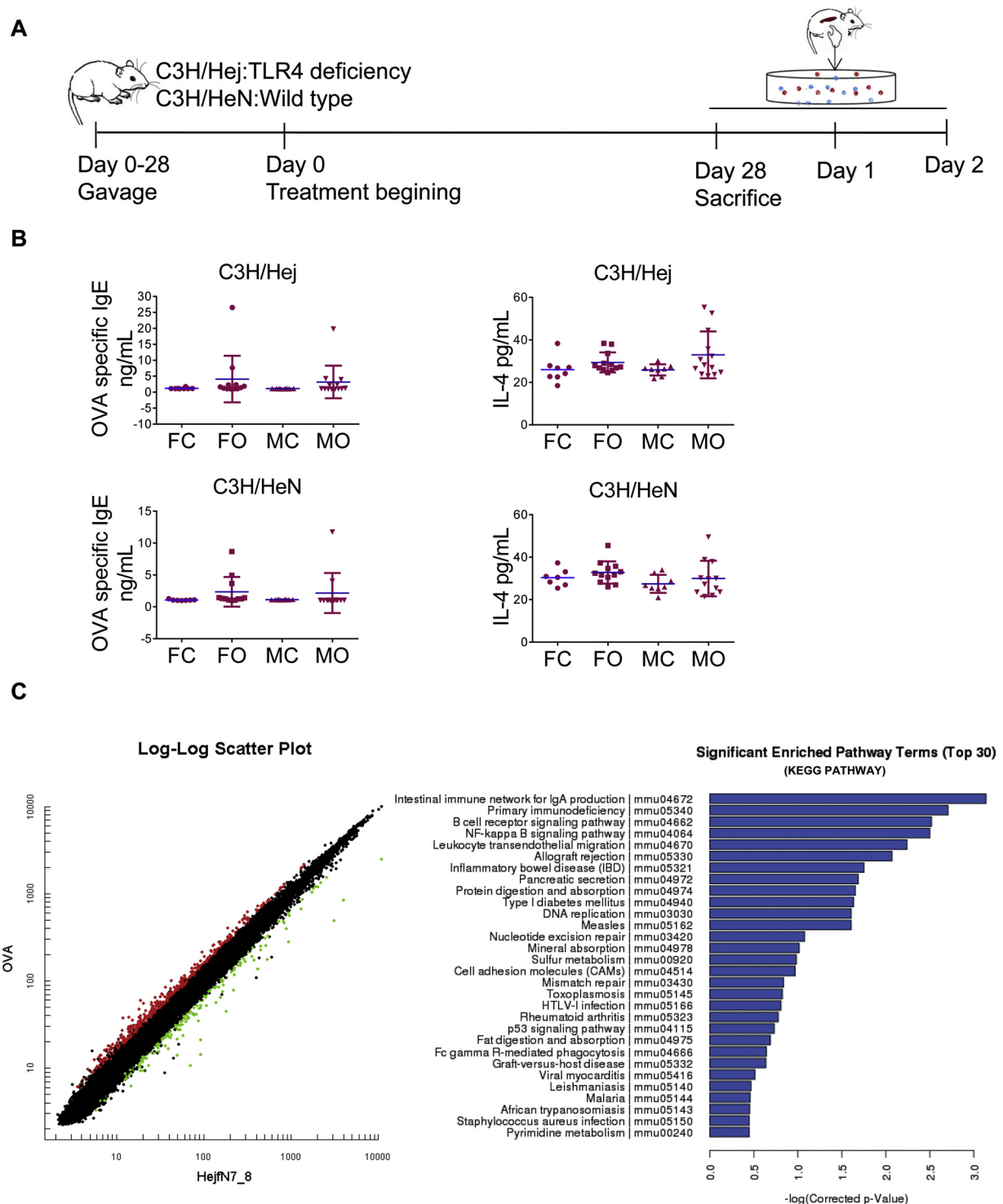


Fig. 1. Affymetrix GeneChip Mouse Genome 430 2.0 Array. (A) C3H/HeN and C3H/HeJ mice were gavaged with OVA for 28 consecutive days. The spleen cells were freshly prepared and cultured for 2 days. (B) OVA-specific IgE levels in the serum and IL-4 levels in the cell culture supernatant. (C) Scatter plots and pathway enrichment by transcriptome microarray. (Red dots, up-regulated genes; green dots, down-regulated genes). FC: female control; FO: female OVA; MC: male control; MO: male OVA (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

cells, which were inconsistent with other researchers.

3.5. IL-21 could elevate the expression of *Aicda* partial through *STAT3* in the whole spleen cells and this effect could be enhanced by IL-17A

To further investigate the relation between IL-21, IL-17A or IL-17B with *Aicda*, the whole spleen cells from naive mice only cultured with

OVA in the presence and absence of IL-21, IL-17A and IL-17B were performed, and the purified B cells were harvested at the end of the experiments (Fig. 5A). It was found that IL-21 could elevate the expression of *Aicda* about 11 folds compared with the control, and when supplied with both of the IL-21 and IL-17A, the expression of *Aicda* elevated about 15 folds, but IL-17B did not show this effect (Fig. 5B). These data suggested that IL-21 significantly enhanced the

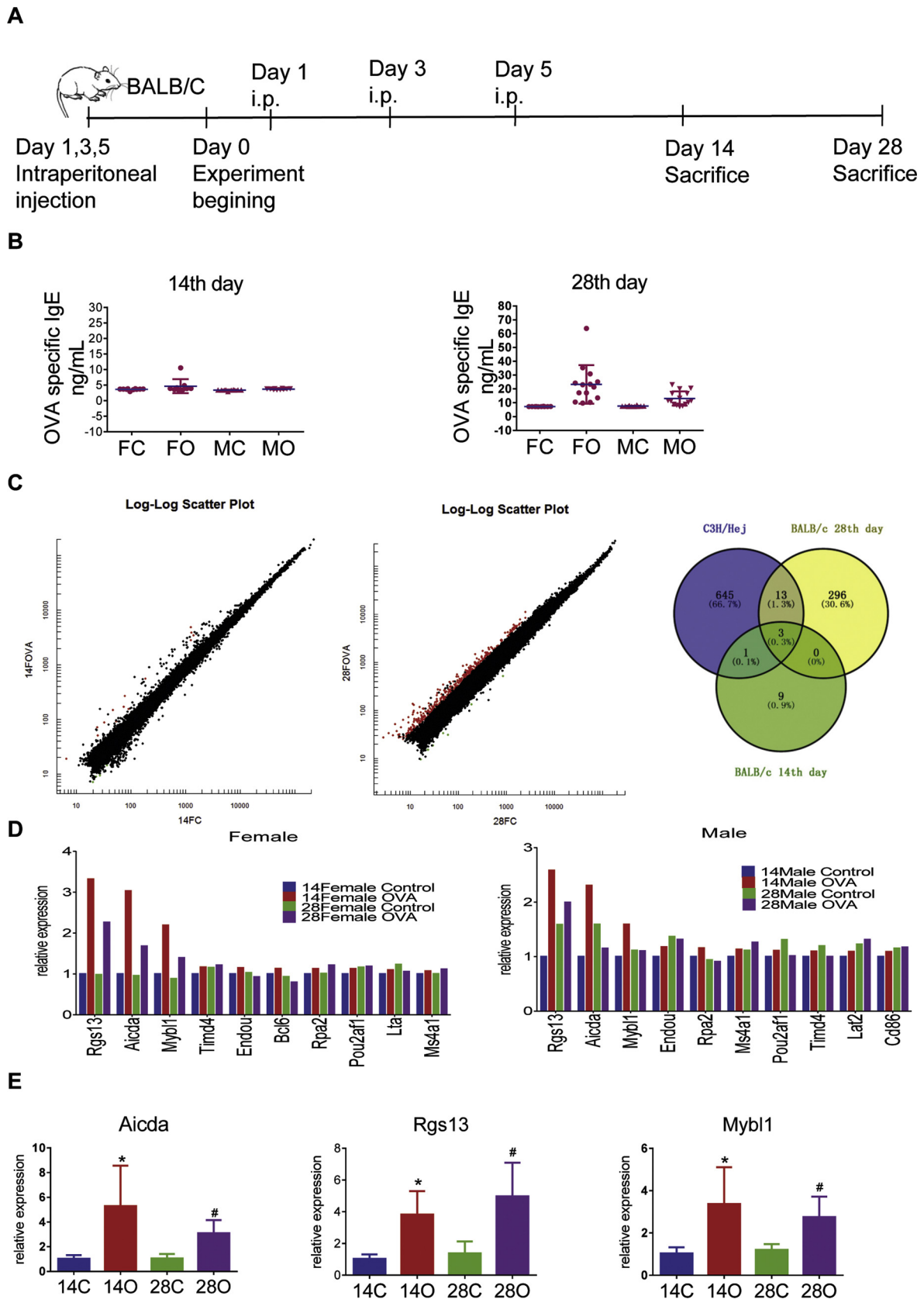


Fig. 2. Agilent mouse lncRNA + mRNA Array V1.0 and QuantiGenePlex Assay. (A) BALB/c mice were injected intraperitoneally with OVA at 2 mg/kg body weight for 3 times(days 1,3,5) and executed at the 14th and 28th day, separately. (B) OVA-specific IgE levels in the serum. (C) Scatter plots of the Agilent mouse lncRNA + mRNA Array V1.0 and overlap between the three microarrays. (D) Results of the QuantiGenePlex Assay. (E) RT-PCR to further confirm the transcription of Aicda, Rgs13 and Mybl1 in the female mice. Data were shown as mean $\pm$ standard deviation. *, $P < 0.05$ compared with the 14day control group; #, $P < 0.05$ compared with the 28day control group. 14C:14 day control; 14O:14 day OVA; 28C: 28 day control; 28O: 28 day OVA.

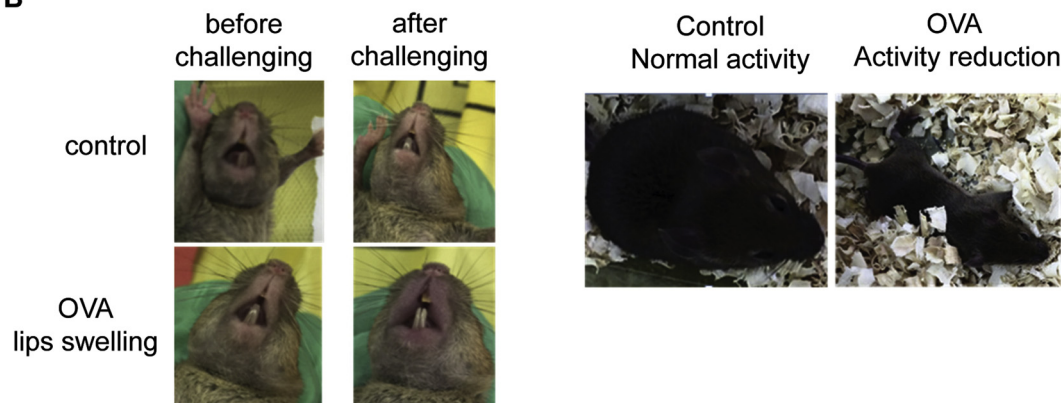
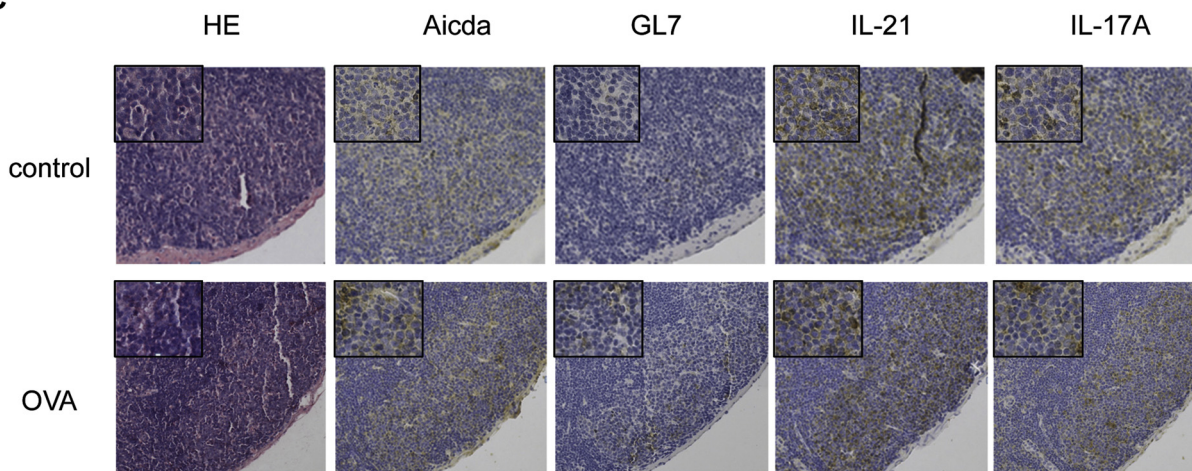
A**B****C**

Fig. 3. High-level expression of Aicda was consistent with the clinical manifestations of allergic mice and co-expressed with IL21 and IL-17A in the Peyer's Patches. (A) C3H/HeN mice were gavaged with OVA for 14 consecutive days and challenged with OVA by intraperitoneal injection at the end of the experiment. (B) Two obvious clinical symptoms including lips swelling and activity reduction were observed in the allergic mice after challenging. (C) Immunostaining of Aicda, GL7, IL-21 and IL-17A in Peyer's Patches of the allergic mice.

expression of Aicda and IL-17A could synergistically facilitate this effect in the cellular microenvironment of lymph gland. Furthermore, when supplied with different doses of IL-21 (12.5, 50 and 200 ng/ml), we found that the promotion effect of IL-21 on the Aicda was in a dose-dependent manner (Fig. 5C).

Previous studies showed that STAT3 is required for IL-21 to induce secretion of IgE in the naive human B cells (Konforte et al., 2009). In this study we investigated whether STAT3 could mediate the promotion effect of IL-21 on the expression of Aicda with the inhibitor of STAT3 (niclosamide). Four control groups were designed in this experiment which added DMF, OVA, IL-21 or OVA + IL-21, respectively, as shown in the Fig. 5D, different doses (5, 10 and 20 μ M) of inhibitor were added and our data showed that the two higher doses can reduce the

expression of Aicda by half. It is very interesting that the promotion of IL-21 on the expression of Aicda was independent of the allergen, because the expression of Aicda in the two controls which added only IL-21 or both of the IL-21 and OVA elevated nearly the same folds, which meant that IL-21 can promote the expression of Aicda by itself after synthesization. Taken together, these data showed that the promotion of IL-21 on the expression of Aicda was dose-dependent and the regulation effect is partial through STAT3 but independent of the allergen.

4. Discussion

In this study, Aicda is significantly over-expressed as a landmark gene at the early stage of food allergy in the OVA-induced allergic mice

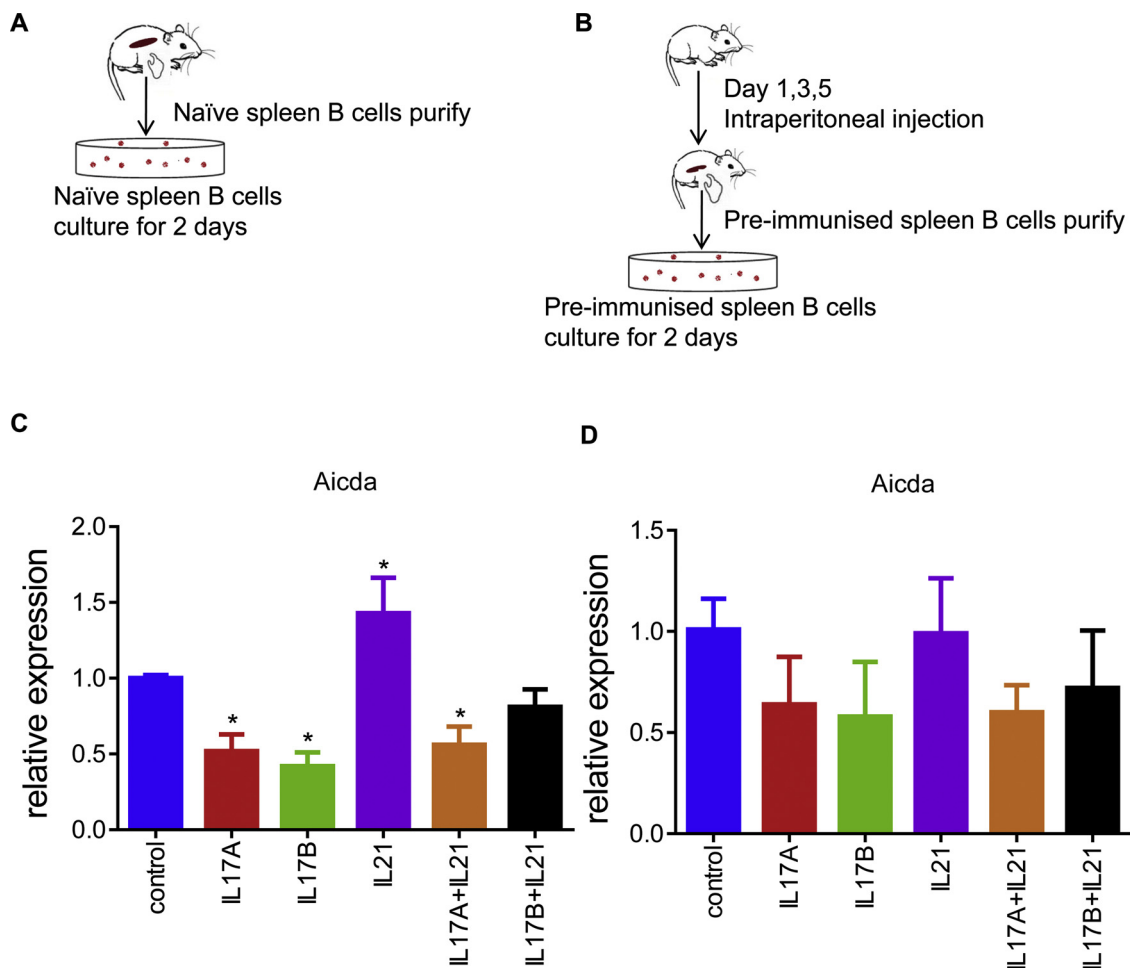


Fig. 4. The promoting effect of IL-21 and IL-17A on the expression of Aicda in the primary spleen B cells is limited. (A) B cells were negatively isolated from the spleens of naive mice (the mice that did not contact allergen previously) and cultured for 2 days. (B) The mice were pre-immunised by intraperitoneal injection with OVA and then purified B cells were isolated from the spleens at the 8th day and cultured for 2 days. (C) Aicda expression in the naive purified B cells which were treated with OVA, IL-4 and anti-mouse CD40 mAb with or without IL-21, IL-17A and IL-17B for 48 h. (D) Aicda expression in the pre-immunised purified B cells which were treated with OVA, IL-4 and anti-mouse CD40 mAb with or without IL-21, IL-17A and IL-17B for 48 h. Data were shown as mean $\pm$ standard deviation. *, $P < 0.05$ compared with the control group.

models. Aicda and IL-21 are highly expressed in the lymph gland of allergic mice and IL-21 can enhance the expression of Aicda partially through activating STAT3. And interestingly, we found that IL-17A can cooperate with IL-21 to significantly elevate Aicda expression though it only has a limited effect by itself.

In food allergy, secretion of IgE is a strictly regulated process, as evidenced by the fact that IgE is 10^4 to 10^5 times lower than IgG and IgA in the serum, but abnormal IgE production can cause severe clinical symptoms (Waldmann et al., 1976). IgE antibody is generated through CSR and can be further modified by SHM, it has been proved that both of them can be mediated by the enzyme Aicda (Chandra et al., 2015; De Silva and Klein, 2015; Klein and Dalla-Favera, 2008; Muramatsu et al., 2000). In this study, we built two animal models of food allergy and the transcriptomic analysis showed that Aicda is significantly over-expressed as a landmark gene in the OVA-induced allergic mice.

The induction of different antibodies in B cells are dependent on two classical signals, the major inducing stimuli include engagement of CD40 by CD154 and cross-linking of the BCR, with specific cytokines synergize to activate the promoter of the relevant immunoglobulin isotype (Kemeny, 2012; Zan and Casali, 2013). IL-4 and IL-21 alone or synergistic are shown to promote IgE production in human B cells (Avery et al., 2008; Carty, 1986; McGuire et al., 2015). In our study we examined the expression of Aicda, IL-21 and IL-17A in the Peyer's Patches of OVA-induced allergic mice, our data showed that they were

all co-expressed in the lymph nodes, among them, Aicda and IL-21 were highly expressed compared to the control. And we found that when activated with OVA, anti-CD40, IL-4 and IL-21, the expression of Aicda in the purified B cells was only slightly elevated. However, the expression of Aicda was dramatically elevated more than 10-folds when the whole spleen cells was only stimulated by IL-21, this effect was dose-dependent and independent of allergen.

Next, we found that when cultured with the whole spleen cells, IL-17A could only slightly increase the expression of Aicda, and in the purified B cells, IL-17A turned to reduce it, which were inconsistent with previous studies (Baek et al., 2014; Milovanovic et al., 2010; Nakajima et al., 2014). But when IL-17A and IL-21 were added simultaneously, the expression of Aicda reached 15 folds compared to the control group in the whole spleen cells, suggesting that IL-17A had a strong synergistic effect with IL-21 to induce Aicda. Previous studies have shown that both of IL-21 and IL-17A can regulate IgE generation by activating STAT3 (Konforte et al., 2009; Kwakkenbos et al., 2016). In our study, STAT3 inhibitor can reduce Aicda expression by half when stimulated with IL-21 which is consistent with other studies.

In summary, our data showed that Aicda is the highest expressed gene in the allergic animals, IL-21 facilitated Aicda expression and further enhanced by IL-17A through STAT3, suggesting that IL-21 and IL-17A can play important role in the development of food allergy. However, IL-21 showed very little promotion effect on the Aicda

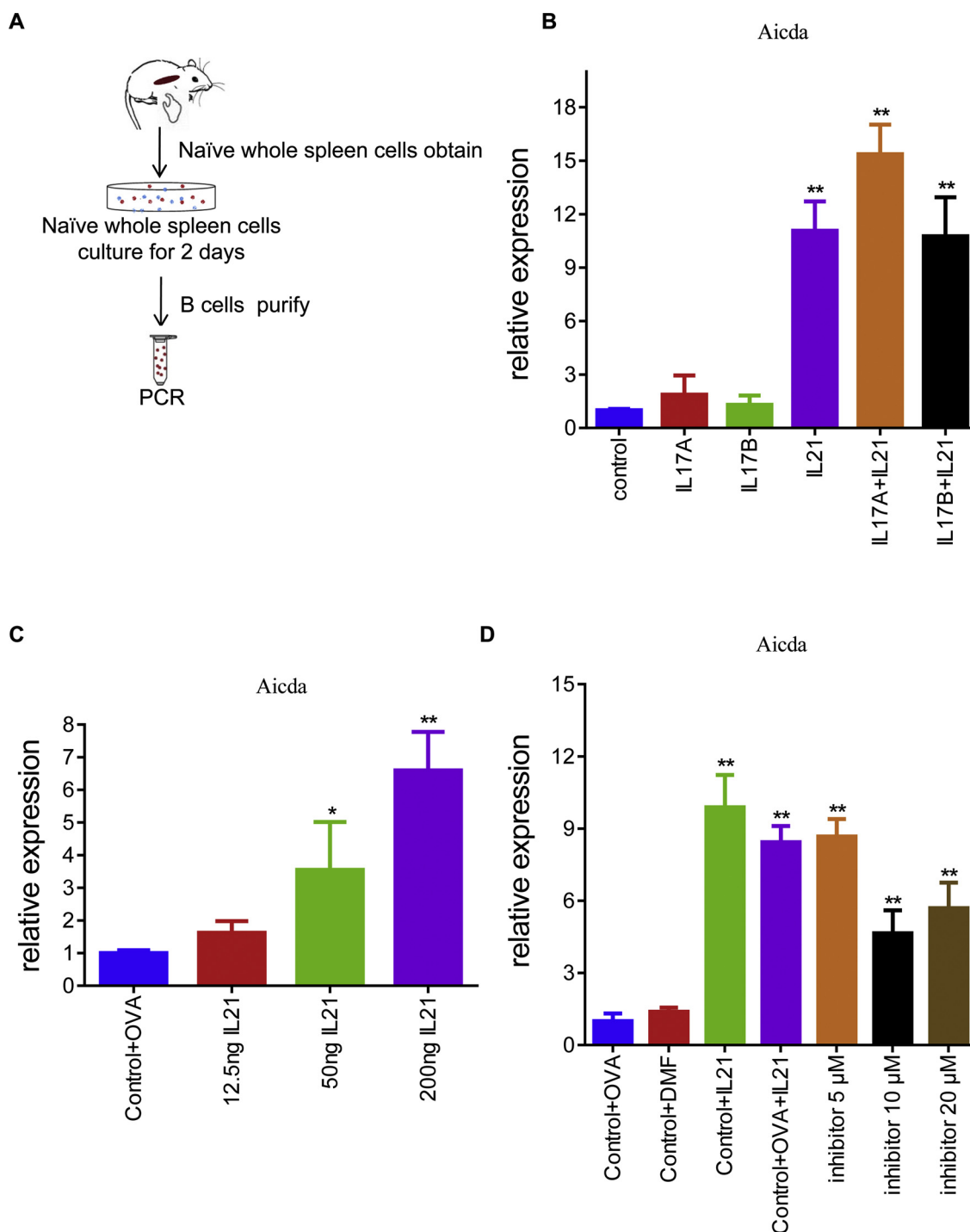


Fig. 5. The promotion of IL-21 on the expression of Aicda was dose-dependent and partial through STAT3 but independent of allergen. (A) The whole spleen cells (including all sorts of cells of spleen) from naïve mice were cultured for 2 days and then B cells were purified to carry out PCR analysis. (B) Aicda expression in the whole spleen cells which were treated with OVA in the presence and absence of IL-21, IL-17A and IL-17B for 48 h. (C) Aicda expression in the whole spleen cells which were treated with OVA and different concentrations of IL-21 for 48 h. (D) Aicda expression in the whole spleen cells which were treated with DMF, OVA and/or IL-21 with different concentrations of STAT3 inhibitor (5,10,20 μ M) for 48 h. Data were shown as mean $\pm$ standard deviation. *, $P < 0.05$ compared with the control group (added OVA only); **, $P < 0.01$ compared with the control group (added OVA only).

expression in the purified B cells, indicating the involvement of other stimuli in the process of OVA-induced allergy, which warrant to further investigation.

Declaration of Competing Interest

All authors declare no conflict of interest.

Acknowledgment

This study was supported by Grants from the National Major Special Project for the Development of Genetically Modified Organisms (2016ZX08011005).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.molimm.2019.08.012>.

References

- Avery, D.T., Ma, C.S., Bryant, V.L., Santner-Nanan, B., Nanan, R., Wong, M., Fulcher, D.A., Cook, M.C., Tangye, S.G., 2008. STAT3 is required for IL-21-induced secretion of IgE from human naive B cells. *Blood* 112, 1784–1793.
- Baek, S.Y., Lee, J., Lee, D.G., Park, M.K., Lee, J., Kwok, S.K., Cho, M.L., Park, S.H., 2014. Ursolic acid ameliorates autoimmune arthritis via suppression of Th17 and B cell differentiation. *Acta Pharmacol. Sin.* 35, 1177–1187.
- Bogh, K.L., van Bilsen, J., Glogowski, R., Lopez-Exposito, I., Bouchaud, G., Blanchard, C., Bodinier, M., Smit, J., Pieters, R., Bastiaan-Net, S., de Wit, N., Untersmayr, E., Adel-Patient, K., Knippels, L., Epstein, M.M., Noti, M., Nygaard, U.C., Kimber, I., Verhoeckx, K., O'Mahony, L., 2016. Current challenges facing the assessment of the allergenic capacity of food allergens in animal models. *Clin. Transl. Allergy* 6, 21.
- Boyce, J.A., Assa'ad, A., Burks, A.W., Jones, S.M., Sampson, H.A., Wood, R.A., Plaut, M., Cooper, S.F., Fenton, M.J., Arshad, S.H., Bahna, S.L., Beck, L.A., Byrd-Bredbenner, C., Camargo Jr, C.A., Eichenfield, L., Furuta, G.T., Hanifin, J.M., Jones, C., Kraft, M., Levy, B.D., Lieberman, P., Luccioli, S., McCall, K.M., Schneider, L.C., Simon, R.A., Simons, F.E., Teach, S.J., Yawn, B.P., Schwaninger, J.M., Panel, N.I.-S.E., 2010. Guidelines for the diagnosis and management of food allergy in the United States: summary of the NIAID-Sponsored expert panel report. *J. Allergy Clin. Immunol.* 126, 1105–1118.
- Carty, R.L.Ca.J., 1986. A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-gamma. *J. Immunol.* 136, 949–954.
- Chandra, V., Bortnick, A., Murre, C., 2015. AID targeting: old mysteries and new challenges. *Trends Immunol.* 36, 527–535.
- De Silva, N.S., Klein, U., 2015. Dynamics of B cells in germinal centres. *Nat. Rev. Immunol.* 15, 137–148.
- Jérôme Pène, J.-F.G., Lécart, Sandrine, Drouet, Elodie, Guglielmi, Paul, Boulay, Vera, Delwail, Adriana, Foster, Don, Lecron, Jean-Claude, Yssel, Hans, 2004. Cutting edge: IL-21 is a switch factor for the production of IgG 1 and IgG3 by human B cells. *J. Immunol.* 172, 5154–5157.
- Kemeny, D.M., 2012. The role of the T follicular helper cells in allergic disease. *Cell. Mol. Immunol.* 9, 386–389.
- Klein, U., Dalla-Favera, R., 2008. Germinal centres: role in B-cell physiology and malignancy. *Nat. Rev. Immunol.* 8, 22–33.
- Kobayashi, S., Haruo, N., Sugane, K., Ochs, H.D., Agematsu, K., 2009. Interleukin-21 stimulates B-cell immunoglobulin E synthesis in human beings concomitantly with activation-induced cytidine deaminase expression and differentiation into plasma cells. *Hum. Immunol.* 70, 35–40.
- Konforte, D., Simard, N., Paige, C.J., 2009. IL-21: an executor of B cell fate. *J. Immunol.* 182, 1781–1787.
- Kulis, M., Wright, B.L., Jones, S.M., Burks, A.W., 2015. Diagnosis, management, and investigational therapies for food allergies. *Gastroenterology* 148, 1132–1142.
- Kwakkenbos, M.J., van Helden, P.M., Beaumont, T., Spits, H., 2016. Stable long-term cultures of self-renewing B cells and their applications. *Immunol. Rev.* 270, 65–77.
- Lack, G., 2008. Epidemiologic risks for food allergy. *J. Allergy Clin. Immunol.* 121, 1331–1336.
- Lajoie, S., Lewkowich, I., Herman, N.S., Sproles, A., Pesce, J.T., Wynn, T.A., Grusby, M.J., Hamid, Q., Wills-Karp, M., 2014. IL-21 receptor signalling partially mediates Th2-mediated allergic airway responses. *Clin. Exp. Allergy* 44, 976–985.
- Liu, T., Navarro, S., Lopata, A.L., 2016. Current advances of murine models for food allergy. *Mol. Immunol.* 70, 104–117.
- McGuire, H.M., Vogelzang, A., Warren, J., Loetsch, C., Natividad, K.D., Chan, T.D., Brink, R., Batten, M., King, C., 2015. IL-21 and IL-4 collaborate to shape T-Dependent antibody responses. *J. Immunol.* 195, 5123–5135.
- Milovanovic, M., Drozdenko, G., Weise, C., Babina, M., Worm, M., 2010. Interleukin-17A promotes IgE production in human B cells. *J. Invest. Dermatol.* 130, 2621–2628.
- Muramatsu, M., Kinoshita, K., Fagarasan, S., Yamada, S., Shinkai, Y., Honjo, T., 2000. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* 102, 553–563.
- Nakajima, S., Kitoh, A., Egawa, G., Natsuaki, Y., Nakamizo, S., Moniaga, C.S., Otsuka, A., Honda, T., Hanakawa, S., Amano, W., Iwakura, Y., Nakae, S., Kubo, M., Miyachi, Y., Kabashima, K., 2014. IL-17A as an inducer for Th2 immune responses in murine atopic dermatitis models. *J. Invest. Dermatol.* 134, 2122–2130.
- Rona, R.J., Keil, T., Summers, C., Gislason, D., Zuidmeer, L., Sodergren, E., Sigurdardottir, S.T., Lindner, T., Goldhahn, K., Dahlstrom, J., McBride, D., Madsen, C., 2007. The prevalence of food allergy: a meta-analysis. *J. Allergy Clin. Immunol.* 120, 638–646.
- Suto, A., Nakajima, H., Hirose, K., Suzuki, K., Kagami, S., Seto, Y., Hoshimoto, A., Saito, Y., Foster, D.C., Iwamoto, I., 2002. Interleukin 21 prevents antigen-induced IgE production by inhibiting germ line C(epsilon) transcription of IL-4-stimulated B cells. *Blood* 100, 4565–4573.
- Waldmann, T.A., Iio, A., Ogawa, M., McIntyre, O.R., Strober, W., 1976. The metabolism of IgE. Studies in normal individuals and in a patient with IgE myeloma. *J. Immunol.* 117, 1139–1144.
- Wang, J.H., New, J.S., Xie, S., Yang, P., Wu, Q., Li, J., Luo, B., Ding, Y., Druey, K.M., Hsu, H.C., Mountz, J.D., 2013. Extension of the germinal center stage of B cell development promotes autoantibodies in BXD2 mice. *Arthritis Rheum.* 65, 2703–2712.
- Zan, H., Casali, P., 2013. Regulation of Aicda expression and AID activity. *Autoimmunity* 46, 83–101.