

IL-21 Induces Inhibitor of Differentiation 2 and Leads to Complete Abrogation of Anaphylaxis in Mice¹

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IL-21 exerts pleiotropic immunomodulatory activities on a variety of target cells including B cells that undergo class switch recombination (CSR) to IgE. In this study, we examined whether IgE-mediated systemic anaphylaxis was controlled by in vivo administration of IL-21 using the peanut allergy model in mice and investigated the molecular mechanisms underlying the IL-21-induced regulation of IgE. The anaphylactic reaction was completely abolished by the administration of recombinant mouse IL-21 or an IL-21 expression plasmid in terms of the change of body temperature and anaphylactic symptoms. The recombinant mouse IL-21 treatment remarkably suppressed IgE CSR in splenic B cells, resulting in significant decrease in serum concentrations of total as well as allergen-specific IgE. In the meanwhile, IL-21 provoked B cells in normal as well as allergic mice to express the inhibitor of differentiation 2 (Id2) gene that was shown to be crucially involved in the regulation of the activation-induced cytidine deaminase and IgE CSR. Moreover, mice genetically deficient for Id2 were completely unsusceptible to IL-21-induced prevention of IgE CSR and anaphylaxis. The present study strongly suggests that IL-21 is capable of regulating systemic allergic reactions by inducing the transcriptional regulator Id2, and the cytokine may be useful for clinical intervention for allergic diseases including anaphylaxis. *The Journal of Immunology*, 2007, 179: 8554–8561.

Immunoglobulin E (IgE) plays central roles in the pathogenesis of many allergic diseases. In sensitized individuals, IgE Abs are produced at high levels and bind to the high-affinity FcεRs on mast cell and basophil surfaces, leading to release of preformed and newly synthesized mediators that initiate immunologic cascades and inflammatory reactions. If the IgE production can be artificially controlled, it may become an ideal strategy to treat allergy. Until now, therapeutic means that suppress IgE production have not been clinically available, although neutralization of pre-existing IgE has been applied to patients with severe allergic asthma (1). Among the allergic diseases, anaphylaxis is the most severe and life-threatening syndrome. Susceptible patients show drastic systemic symptoms including cardiovascular, respiratory, and neurological perturbations after intake of specific allergens, such as particular food, insect venom, or medication. Among them, peanut is one of the most common food Ags that cause anaphylaxis (2).

IL-21 is a member of the 4-helix bundle type I cytokine family with significant homology to IL-2, IL-4, and IL-15 (3). The IL-21R complex is composed of IL-21R and the common γ-chain, the latter playing critical roles in the signal transduction pathway (4).

IL-21 is secreted by activated T cells and regulates proliferation, differentiation, and activation of NK cells, Th1, Th2, Th17, CTL, dendritic cells, and macrophages (3, 5–8). Intriguingly, IL-21 exerts various activities on B cells, including differentiation (9–11), growth promotion (12, 13), growth suppression (12, 14), and apoptosis (12, 15), depending on the conditions of stimulation.

IL-21-mediated regulation of IgE also remains enigmatic. Studies on IL-21R (16) and IL-21 (17) gene knockout (KO)⁴ mice suggested that the lack of IL-21 signaling lead to overproduction of IgE. Gain-of-function studies in vitro (18–22) and in vivo (18) reported that exogenous IL-21 may either suppress or enhance IgE production. Suto et al. (18) reported that IL-21 may act directly on IL-4-stimulated B cells leading to inhibition of IgE class switch recombination (CSR) and subsequent reduction of IgE production. Moreover, IL-21 elevated apoptosis in IgE producing B (B_ε) cells that had been induced by soluble CD40L plus IL-4 (22). Other reports suggested that IL-21 may indirectly interfere with IgE production through IFN-dependent (21) or -independent (19) mechanisms rather than directly act on B cells. IL-21 may even up-regulate IgE production in vitro depending on the culture conditions (20, 21).

In this context, it is intriguing to investigate whether IL-21 affects IgE production and allergic symptoms in vivo. We recently found that administration of recombinant mouse IL-21 (rmIL-21) into the nostril of allergic-rhinitis mice significantly inhibited the nasal-allergic reactions as well as IgE CSR in nasal-associated lymphoid tissues (23), although molecular basis for the linkage between IL-21 signal and IgE regulation remains to be elucidated.

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⁴ Abbreviations used in this paper: KO, knockout; CPE, crude peanut extract; CSR, class switch recombination; B_ε, IgE producing B; Id, inhibitor of differentiation; rmIL, recombinant mouse IL; i.g., intragastric; sGLT, germline Cε transcript; AID, activation-induced cytidine deaminase; WT, wild type; Pax5, paired box protein 5; HLH, helix-loop-helix.

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In this study, we treated mice in an anaphylactic model with IL-21 and examined its effects on the systemic allergic symptoms, as well as molecular mechanisms of the IL-21-induced regulation of IgE production.

Materials and Methods

Cells preparation, recombinant cytokines, and crude peanut extract (CPE)

Splenic B cells were enriched using anti-B220 Ab-coated magnetic beads (Miltenyi Biotec) and MACS. More than 95% of the population was B220⁺ as confirmed by flow cytometric analysis using anti-B220 Ab (eBioscience). rmIL-4 and rmIL-21 were purchased from R&D Systems. CPE was prepared as described elsewhere (24). Briefly, the beans of raw peanut were crushed and degreased by Hexane. The extract was dry-roasted at 170°C for 10 min and immersed in 1 M NaCl/20 mM sodium phosphate/8 M urea over night at 4°C. After centrifugation, supernatant was collected and used as CPE. Total protein concentration was measured using a BCA protein assay kit (Promega).

In vivo studies

All the animal experiments were approved by the institutional committee of the Kyoto Prefectural University of Medicine. Female AKR/J mice were purchased from Shimizu Laboratory Suppliers. Inhibitor of differentiation 2 (Id2) KO mice and their littermates (129/Sv genetic background) were maintained under specific pathogen-free conditions in the institutional animal facility and used at 7–10 wk of age. Anaphylactic model was established as previously described (24). Briefly, mice were given intragastric (i.g.) administrations with 1 mg CPE and 10 µg cholera toxin (List Biological Laboratories) via a catheter four times at a weekly interval (days 0, 7, 14, and 21). The mice were also injected i.p. with 0.5 µg CPE and aluminum hydroxide as an adjuvant into the peritoneal cavity on days 1, 8, 15, and 22. On day 28, 10 mg CPE was administered i.g. into the mice using a catheter (challenge). Anaphylactic score was evaluated as described (24) with slight modifications (Table I). The i.v. gene transfection was performed as described previously (25, 26). Briefly, pGEG.mIL-21 and pGEG.4 plasmids (25, 26) were purified using Qiagen MaxiPrep Endo-Free kits (Qiagen). Mice were i.v. injected via the tail vein with 25 µg DNA dissolved in 1.6 ml saline. The injection was completed within 5 s. The procedure enables abundant transgene expression predominantly in the liver, from which soluble transgene product is released to systemic circulation (27–29). To administrate cytokine, 0.5 µg rmIL-21 was injected into the peritoneal cavity of mice.

RT-PCR and real-time RT-PCR

Total RNA was extracted from B220⁺ B cells by the guanidinium acid phenol method. After reverse-transcription, aliquots of cDNA were subjected to RT-PCR using a pair of Cε-specific upstream (5'-gcacaggggcaagaat-3') and downstream (5'-cgttgatgatgagat-3') primers (30), a pair of Cγ1-specific upstream (5'-cagatcttgatgcatcatcag-3') and downstream (5'-gattcaacttgcgcatcatctaggc-3') primers (30), or a pair of GAPDH gene-specific sense (5'-cgtcccgtagacaaatggt-3') and anti-sense (5'-ccctgtgctgtagcgtat-3') primers. PCR conditions are as follows: denaturation at 94°C for 45 s, annealing at 50°C for 45 s and extension at 72°C for 1.5 min for a total of 32 cycles (germline Cε transcript (εGLT)), denaturation at 94°C for 2 min, annealing at 47°C for 2 min and extension at 72°C for 2 min for a total of 30 cycles (germline Cγ1 transcript), and denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 1 min for a

total of 25 cycles (GAPDH). The PCR products were separated by gel electrophoresis through 1.2% agarose gel followed by ethidium bromide staining. Real-time RT-PCR was performed using the 7300 Real Time PCR System (Applied Biosystems). The matching primers and dye probe for the Id2 (Mm00711781-m1), the activation-induced cytidine deaminase (AID; Mm00507774-m1), and β-actin (Mm00607939-m1) were purchased from Applied Biosystems. The εGLT sequence was amplified using Quantitect SYBR Green PCR kit (Qiagen). RNA levels were quantified by RQ software (Applied Biosystems).

ELISA

Each class and subclass of Ig as well as histamine were measured using a mouse total IgE ELISA kit (Morinaga Institute of Biological Science), total IgG1 ELISA kit (Alpha Diagnostic International), total IgG2a ELISA kit (Alpha Diagnostic International), total IgG3 ELISA kit (Alpha Diagnostic International), and histamine ELISA kit (Beckman Coulter). CPE-specific IgE was titrated by coating microtiter plates with 2 µg/ml CPE at 4°C overnight. After blocking, diluted serum samples were added and incubated for 1 h at room temperature. The plates were washed and incubated with biotinylated anti-mouse IgE Ab (Bethyl Laboratories) followed by an addition with streptavidin-HRP (Rockland) and tetramethylbenzidine substrate. Absorption at 450 nm was measured and analyzed by the Microplate Manager (Sumitomo Pharma).

Western blot analysis

Cells were extracted using Qproteome Mammalian Protein prep kit (Qiagen). Each aliquot of the extract containing 30 µg soluble protein was loaded onto 10% SDS polyacrylamide gel, and after electrophoresis the gels were blotted onto polyvinylidene difluoride membrane, which was subsequently probed with anti-mouse Id2 (C-20, Santa Cruz Biotechnology) and anti-β-actin (Poly6221, BioLegend) Abs. The signals were developed using chemiluminescent Western blot immunodetection kit (Invitrogen Life Technologies), and quantified using a Versa Doc imaging system (Bio-Rad) and Quantity One software (version 4.5.1; Bio-Rad).

Flow cytometric analysis

Cells were permeabilized using Cytofix/Cytoperm solution (BD Pharmingen) and incubated with anti-mouse Id2 Ab (C-20; Santa Cruz Biotechnology). After washing, cells were incubated with FITC-anti-rabbit IgG Ab (Beckman Coulter) followed by cytometric analysis using a FACSCalibur (BD Immunocytometry Systems).

Statistical evaluation

Significant differences between anaphylactic scores were analyzed by the Mann-Whitney *U* test. For other comparisons, Student's *t* test was used. Results are expressed as means ± SD.

Results

IL-21 totally abrogates anaphylactic reactions in peanut allergic mice

The peanut allergy model was established in mice by repetitive priming with CPE. They also received by i.v. transfection a control plasmid (pGEG.4) or an IL-21 gene expression vector (pGEG.mIL-21; (25)). After i.g. challenge with the allergen, the control mice showed a significant fall of body temperature, which represents a typical sign of anaphylaxis (Fig. 1A). In sharp contrast, repetitive transfection with the pGEG.mIL-21 completely normalized the change in body temperature. The IL-21 gene-mediated inhibition of anaphylaxis was also confirmed by scoring the anaphylactic symptoms based on the respiratory and peripheral circulatory disturbance, neurological manifestations, as well as survival of the mice (Table I) (24). Although allergic mice given control plasmid developed severe symptoms accompanied with high anaphylactic scores, the scores for the pGEG.mIL-21-treated animals were as low as those for the nonallergic controls (Fig. 1B). Similar results were also obtained in other series of experiments, in which i.p. administration of rmIL-21 also totally abrogated the anaphylactic symptoms in peanut allergy mice (Fig. 1, C and D).

ELISA analyses were performed to assess serological change in control-, allergic-, and rmIL-21-treated allergic mice. Total IgE level was remarkably decreased in sera of rmIL-21-administered

Table I. Scoring of anaphylactic symptoms^a

Score	Symptom
0	No sign of reaction
1	Scratching and rubbing around nose and head
2	Decreased activity with an increasing respiratory rate
3	pilar erecti and/or puffing around the eyes
4	Labored respiration and cyanosis around the mouth and tail
5	Slight or no activity after prodding or tremors and convulsion
6	Death

^a The anaphylactic symptoms were quantified as described (according to Ref. 24).

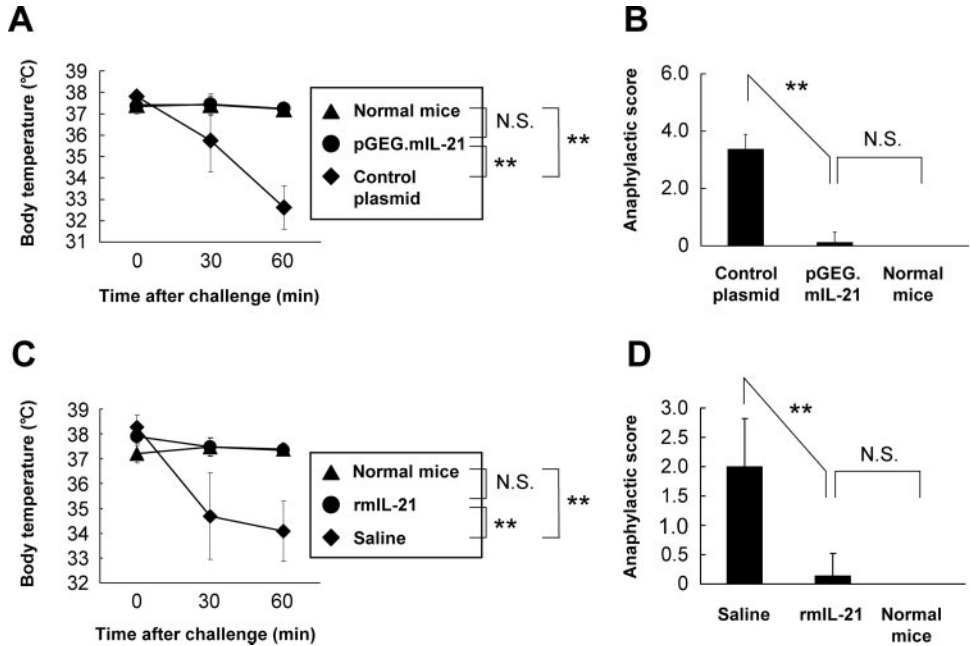


FIGURE 1. IL-21 completely prevented anaphylactic reaction in mice. *A* and *B*, Mice were sensitized with CPE and transfected with 25 μ g mIL-21 expression plasmid (pGEG.mIL-21) or control plasmid (pGEG.4) (days 0, 7, 14, and 21). After i.g. challenge with CPE (day 28), rectal temperature was measured (*A*), whereas anaphylactic symptoms were scored 60 min after the challenge (*B*). *C* and *D*, Mice were sensitized with CPE and administered i.p. with rmIL-21 or saline (days 0, 3, 7, 10, 14, 17, 21 and 24). After CPE challenge on day 28, rectal temperature (*C*) and anaphylactic scores (*D*) were measured as above. $n = 7$ mice per group. **, $p < 0.005$; N.S., Not significant.

group, reaching a comparable level as nonallergic control animals (Fig. 2*A*). CPE-specific IgE was also markedly reduced by IL-21, compared with that in the allergic mice administered with saline

(Fig. 2*B*), whereas serum concentrations of IgG subclasses did not significantly differ between saline and rmIL-21 groups (Fig. 2, *C–E*). In addition, the serum level of histamine was completely

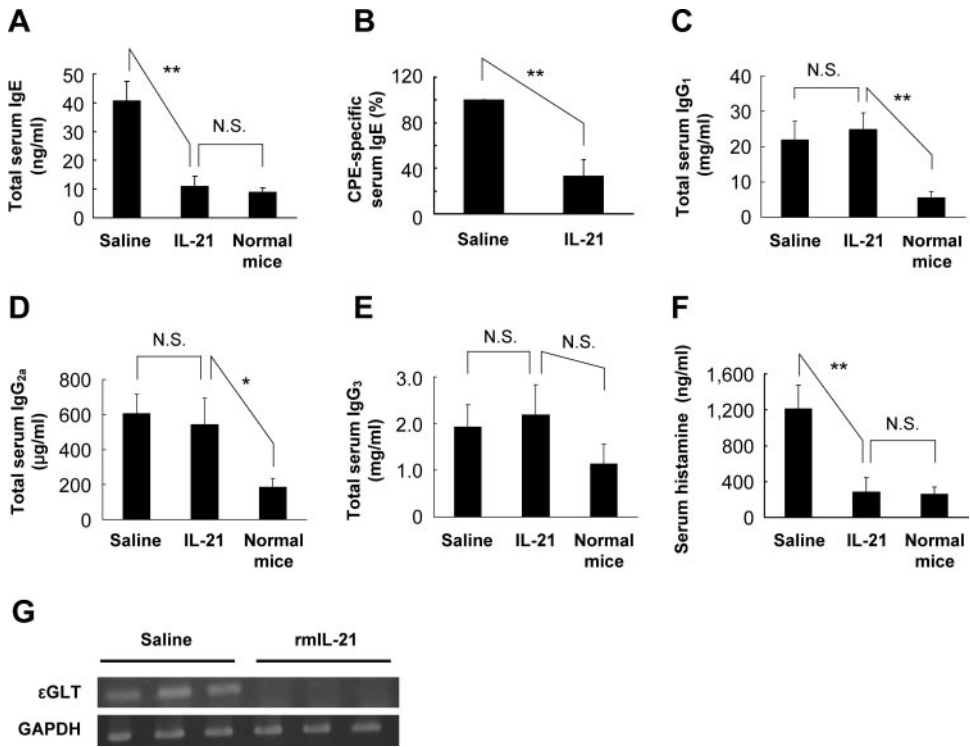


FIGURE 2. IL-21 hampered ϵ GLT and IgE production in vivo in anaphylactic mice. Mice were immunized with CPE and administered i.p. with rmIL-21 or saline as in Fig. 1*C*. On day 28, these mice as well as nonsensitized control mice were challenged i.g. with CPE. Sixty minutes later, mice were sacrificed and the sera were subjected to ELISA to evaluate the concentrations of IgE (*A*), CPE-specific IgE (*B*), IgG₁ (*C*), IgG_{2a} (*D*), IgG₃ (*E*), and histamine (*F*), whereas total RNA was obtained from the splenic B220⁺ B cells and served for RT-PCR using primers for ϵ GLT and GAPDH (*G*). $n = 7$ (*A–F*) and 3 (*G*) mice per group. *, $p < 0.05$; **, $p < 0.005$; N.S., Not significant.

normalized in the rmIL-21-administered group, which showed virtually the same level of the chemical mediator as normal animals (Fig. 2F).

To assess the effect of IL-21 on IgE CSR, splenic B cells were isolated from the animals and ε GLT was detected by RT-PCR. The analysis clearly demonstrated the presence of the transcript in allergic mice that received saline, whereas the expression was very faint, if any, in the B cells from rmIL-21-administered animals (Fig. 2G).

IL-21 evokes Id2 expression in B cells

To investigate the mechanism of the IgE suppression, we performed DNA microarray analysis on cDNA from murine splenic B cells that were cultured with or without rmIL-21. As a result, several genes were significantly induced by IL-21 (data not shown). Among others, we focused on Id2 that we had previously reported as an important regulator of IgE CSR (31). Real-time RT-PCR analysis confirmed that IL-21 provoked B cells to express Id2 (Fig. 3A and data not shown). The Id2 induction was demonstrated even in the presence of IL-4 plus LPS that otherwise provide signals to

promote IgE CSR, albeit at a smaller magnitude (Fig. 3A). It was also found that addition of rmIL-4 dose-dependently counteracted the Id2 induction by rmIL-21 (Fig. 3C). The Western blotting (Fig. 3D) and flowcytometric analysis (Fig. 3E) demonstrated a several fold elevation of Id2 protein in IL-21-stimulated B cells, whereas only faint expression of Id2 was seen in IL-21-nontreated B cells. Also, Id2 mRNA was significantly up-regulated in vivo in splenic B cells of AKR/J mice that received an i.v. injection with pGEG-mIL-21 (Fig. 3F). Id2 was also induced in splenic B cells in allergic mice that received rmIL-21 administration (Fig. 3G).

We further examined the expression levels of AID gene that is essentially required for CSR (32, 33) and that is suppressed by Id2, as we previously reported (34). IL-21 significantly down-modulated AID expression in cultured B cells as demonstrated by DNA microarray analysis (data not shown) and real-time RT-PCR (Fig. 3B and data not shown).

Id2 is required for the IL-21-mediated modulation of IgE CSR

The above data suggested that Id2 may be crucially involved in the suppression of IgE CSR by IL-21. To assess this possibility,

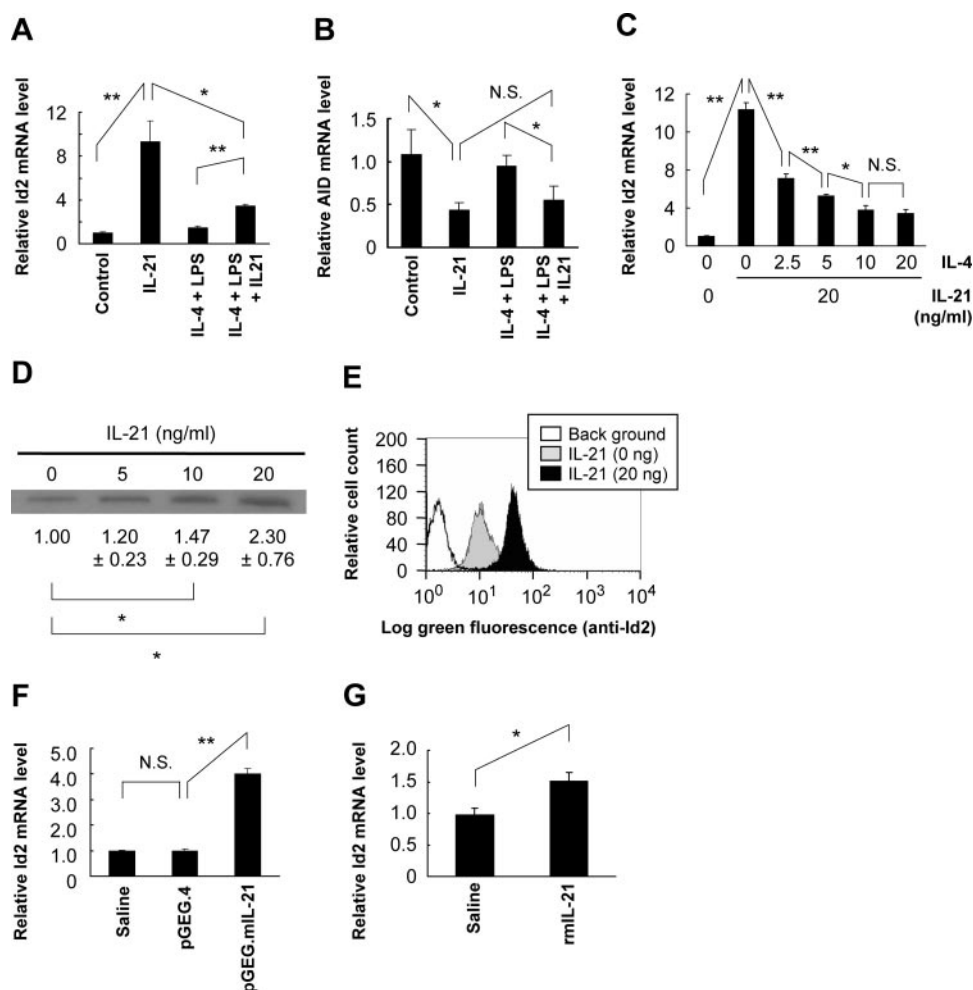


FIGURE 3. IL-21 provoked B cells to express Id2 in vitro and in vivo. *A* and *B*, AKR/J spleen B cells were cultured with/without LPS (10 μ g/ml), rmIL-4 (20 ng/ml), and rmIL-21 (10 ng/ml). Six hours later, total RNA was subjected to real-time RT-PCR. Shown are relative Id2 (*A*) and AID (*B*) mRNA levels vs β -actin. *C*, Spleen B cells were stimulated with the indicated concentrations of IL-4 and IL-21 for 6 h. Shown are relative Id2 mRNA levels vs β -actin evaluated by real-time RT-PCR. *D* and *E*, Immunoblot (*D*) and flowcytometric (*E*) analyses demonstrated Id2 induction in AKR/J splenic B220⁺ B cells cultured with rmIL-21 for 10 h. Numbers below the lanes in *D* indicate the relative signal intensities of the bands. As a control in *E*, rmIL-21-untreated cells were stained with secondary Ab alone (background). *F* and *G*, IL-21 induced Id2 in vivo. Real-time RT-PCR demonstrated significant Id2 mRNA expression in splenic B220⁺ B cells obtained from AKR/J mice 12 h after in vivo transfection with the mIL-21 gene (*F*), while Id2 was also induced in B cells in allergic AKR/J mice given rmIL-21 as in Fig. 2G (*G*). Control mice were injected with saline. Shown are relative Id2 mRNA levels vs β -actin. $n = 3$ mice per group. *, $p < 0.05$; **, $p < 0.005$; N.S., Not significant.

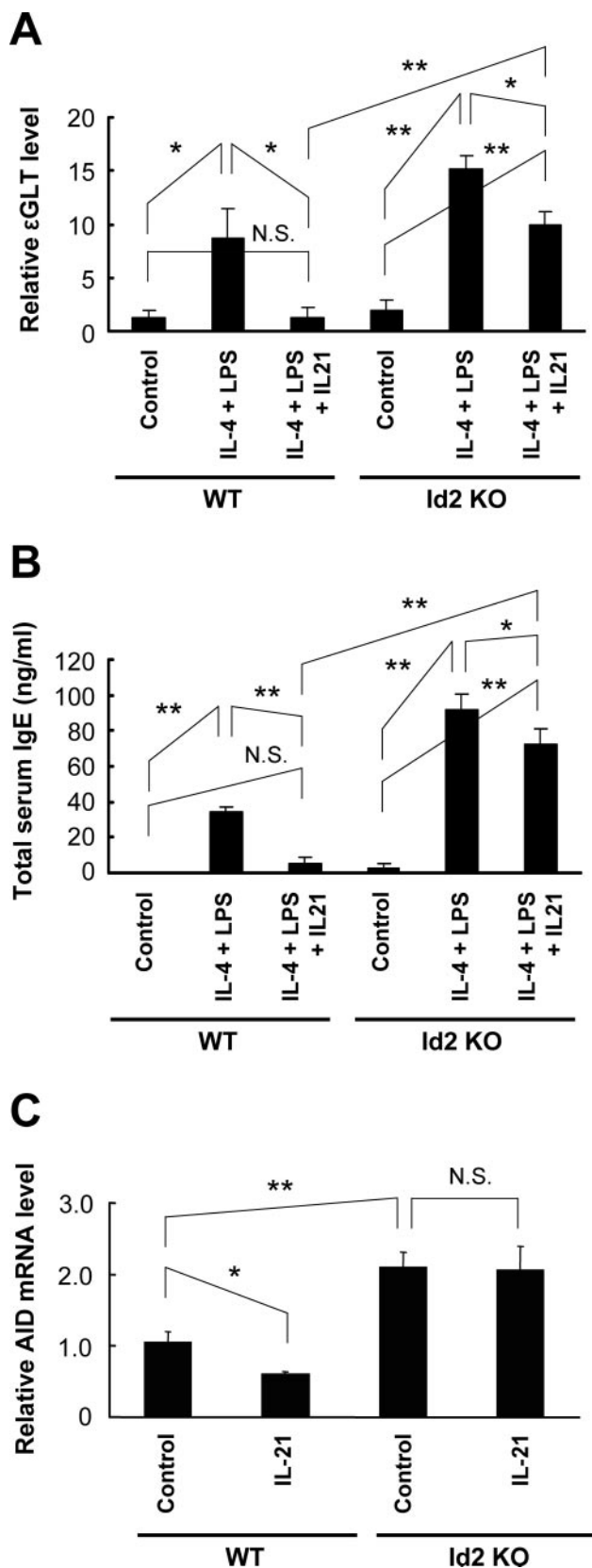


FIGURE 4. Requirement of Id2 in IL-21-mediated suppression of IgE CSR. Splenic B cells were obtained from Id2 KO or WT littermate mice and cultured with/without LPS (10 μ g/ml), mIL-4 (20 ng/ml), and mIL-21 (10 ng/ml). **A**, Sixteen hours later, real-time RT-PCR was performed. Shown are relative ϵ GLT levels vs β -actin. **B**, Seven days later, IgE concentrations in the culture supernatant were measured by ELISA. **C**, Six hours later, real-time RT-PCR was performed. Shown are relative AID levels vs β -actin. $n = 3$ mice per group. *, $p < 0.05$; **, $p < 0.005$; N.S., Not significant.

splenic B cells were collected from Id2-deficient mice or their wild-type (WT) littermates, and after culturing with/without IL-4, LPS, and IL-21, ϵ GLT was estimated. RT-PCR analysis clearly demonstrated that the IL-21-mediated inhibition of ϵ GLT was markedly hampered in the Id2 null B cells (Fig. 4A and data not shown). ELISA analysis revealed that Id2-deficient B cells produced a significant amount of IgE in response to IL-4 plus LPS stimuli (Fig. 4B). Addition of IL-21 only marginally suppressed IgE production by Id2 KO B cells, in striking contrast to the remarkable IL-21-induced reduction of IgE secretion by WT B cells (Fig. 4B).

We also tested whether Id2 is required for the IL-21-mediated suppression of AID. As shown in Fig. 4C, mIL-21 stimuli failed to affect AID expression in Id2 KO B cells, strongly suggesting that IL-21 down-regulates AID through induction of Id2.

Id2 is crucially involved in IL-21-mediated prevention of anaphylaxis

Next, we examined whether Id2 is responsible for IL-21-mediated regulation of the anaphylactic reaction. In the absence of IL-21 administration, Id2 KO mice lost their body temperature as drastically as WT mice (Fig. 5A), and severe anaphylactic symptoms were induced regardless of the Id2 genotype (Fig. 5B). Interestingly, the effects of IL-21 were evident only in WT animals, which were totally protected from the anaphylaxis, whereas the cytokine failed to relieve the systemic allergic reaction of the mice genetically lacking Id2 (Fig. 5, A and B). RT-PCR, real-time RT-PCR, and ELISA analyses also revealed that the Id2 KO mice were resistant to the IL-21-induced suppression of ϵ germ line transcription (Fig. 5C and data not shown) as well as of allergen-specific IgE production (Fig. 5D). These results clearly demonstrate that Id2 is a prerequisite to the suppression of IgE production, and resultant amelioration of anaphylactic reaction, by IL-21 treatment.

Discussion

Although the mechanism of CSR has not been fully elucidated, it has been widely known that the germline IgH transcript and AID play central roles in the CSR. The noncoding GLT across the switch region precedes CSR, and shapes the "transcriptional bubble," in which the unpaired nontemplate strand of DNA is displaced from its complementary strand (35). AID is the only specific factor that is necessary for, and sufficient to, the CSR (32, 33). AID either deaminates cytosines in the ssDNA, leading to subsequent dsDNA breaks (36), or functions as an RNA editor that may be required for yet unidentified recombinase molecule(s) (32, 33). Both AID and ϵ GLT are positively regulated by three transcriptional factors, i.e., paired box protein 5 (Pax5) (34, 37), E2A (38, 39), and STAT6 (40, 41). Pax5 is constitutively expressed throughout the B cell lineage (up until the terminal differentiation into plasma cells) and further up-regulated by CD40 engagement (42). E2A is induced by LPS, IL-4 plus anti-IgM, or IL-4 plus anti-CD40 (43), whereas STAT6 is activated by IL-4/IL-13 signals (44). Id2 interacts with E2A to inhibit its dimerization, resulting in block of this transcriptional factor (39), whereas Pax5 is also suppressed by Id2 (34). Therefore, Id2 is an important regulator that down-regulates IgE CSR through inhibition of Pax5 and E2A (34, 39), and this notion is supported by our previous report that Id2 KO mice show an elevated serum level of IgE but not of other classes of Ig (45). The present findings that Id2 is induced by IL-21 and crucially involved in the IL-21-provoked inhibition of IgE CSR provide the molecular basis for IgE regulation by this cytokine. In the meanwhile, IL-21 has been shown to induce Bcl-6 transcriptional repressor (9), which reportedly inhibits STAT6

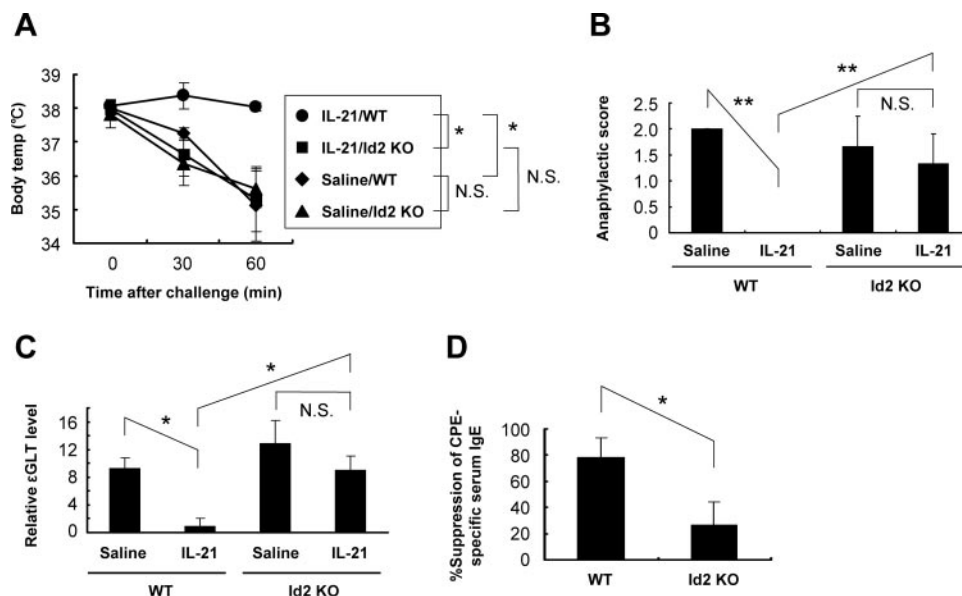


FIGURE 5. IL-21 failed to alleviate anaphylactic symptoms in Id2 KO animals. Id2 KO or WT littermate mice were immunized with CPE and administrated i.p. with rmIL-21 or saline as in Fig. 1C. On day 28, mice were challenged i.g. with CPE. *A* and *B*, Rectal temperature (*A*) and anaphylactic scores (*B*) were evaluated. *C* and *D*, Mice were sacrificed 60 min after the challenge. Total RNA obtained from the splenic B cells was subjected to real-time RT-PCR to evaluate relative ϵ GLT levels vs β -actin mRNA (*C*), whereas CPE-specific serum IgE was evaluated by ELISA (*D*). $n = 3$ mice per group. *, $p < 0.05$; **, $p < 0.005$; N.S., Not significant.

(46), suggesting another pathway of the IL-21-mediated control of IgE, although it remains obscure whether this pathway is also indispensable for IL-21-induced IgE suppression, due to the lack of information on IL-21 effect on allergic Bcl-6 KO mice.

Although AID is essential for CSR to both IgE and IgG, it remains unveiled whether IgE CSR and IgG CSR require comparable levels and duration of AID expression in B cells. This may explain our experimental findings that IL-21-mediated suppression of AID (Fig. 3*B*) was accompanied by reduction of IgE but not IgG (Fig. 2, *A–E*).

CSR to various Ig classes is regulated by multiple cytokines, including IFN- γ , IL-4, and TGF- β (reviewed in Ref. 47). In mice, IL-4 enhances CSR to IgG1 and IgE by inducing γ_1 and ϵ GLT in activated B cells, while IFN- γ accelerates IgG2a CSR, and TGF- β is a switch factor for IgG2b and IgA (47). Deenick et al. (48) stimulated murine B cells with various combinations of these cytokines in vitro and proposed that the cytokines function in a hierarchical fashion: IFN- γ is dominant over IL-4, and both IL-4 and IFN- γ are dominant over TGF- β (48). IL-21 suppressed IgE CSR that was otherwise induced by IL-4 plus LPS ((18); Fig. 3*B* and 4), suggesting IL-21 as an important cytokine among the CSR regulating factors. Our present findings that IL-4 counteracted the Id2-inducing activity of IL-21 and vice versa strongly suggest that the balance between IL-4 and IL-21 may determine the expression level of Id2 that subsequently determines the rate of IgE CSR (Fig. 3, *A* and *C*, and 4). The functional ambivalence of IL-4 and IL-21 is quite intriguing because strong sequence homology is seen between these two cytokine genes as well as between the IL-4 receptor α -chain and IL-21R genes that locate adjacent to each other and share structural similarity. Skewing of the balance between IL-4 and IL-21 signals could etiologically be associated with allergic hypersensitivities. Hecker et al. (49) analyzed genetic polymorphisms in the human IL-21R gene and found that the presence of the T-83C variant was associated with elevated IgE serum levels in healthy and, to a greater extent, in atopic female individuals. Supplementation of the IL-21 signal may be a reasonable strategy

to correct Id2 expression and in turn alleviate allergic symptoms in allergic patients.

Previous studies suggested that exogenous IL-21 may either elevate or suppress IgG1 production depending on experimental conditions (9, 18). In our allergic mice model, IL-21 administration in vivo did not affect serum levels of IgG subclasses (Fig. 2, *C–E*).

Id proteins contain the helix-loop-helix (HLH) motif but lack a DNA binding domain (39, 50). They interact with the E proteins, a subclass of the basic HLH transcriptional factors, and block the ability of the E proteins to bind to their target DNA sequences (E-box) (51). Id proteins thus antagonize the transactivation functions of the E proteins, which otherwise regulate many aspects of hematopoiesis as well as lymphocyte proliferation and survival through modulating gene expression programs. Id proteins are positive regulators of cell-cycle progression and oncogenesis, as well as inhibitors of cell differentiation, while they also exert negative control on cell fate decisions mediated by basic HLH factors (50, 52, 53). Four Id genes have been identified in mammals, i.e., Id1 to 4. We and others previously reported several developmental anomalies in mice genetically deprived of Id2. They lacked lymph nodes and Peyer's patches, whereas the splenic architecture remained mostly intact exhibiting distinct T and B cell compartments (54). The development of NK cells, CD8⁺ dendritic cells, Langerhans cells, and intraepithelial lymphocytes were obstructed, whereas $\alpha\beta$ T cells developed normally in Id2 null animals (45, 54–57). Id2 was shown to contribute to the regulation of the CD8⁺ T cell immune response by preventing apoptosis in activated CD8⁺ effector T cells, although initial Ag responses of CD8⁺ T cells were independent of Id2 (58). With respect to B cells, we previously found that the balance between Id2 and Pax5 was pivotal for the regulation of AID gene expression (34). We also reported that Id2 negatively regulated IgE CSR (31), whereas Id2-deficient mice showed elevated IgE levels in their sera (45). The regulation of IgE may imply a sort of defense mechanism in which Id2 may protect the host from potentially harmful overproduction

of IgE after infection with helminthic parasites (39). Although transcriptional regulation of Id2 has not been fully unveiled, we previously reported that TGF- β 1 induces Id2 expression in B cells (31).

IL-21 was shown to induce apoptosis in cultured murine B cells (12, 15). Harada et al. (22) recently demonstrated that IL-21 provoked B ϵ cells to express the proapoptotic Bmf gene and undergo apoptosis in vitro. They also clearly demonstrated that *Mycobacterium bovis* bacillus Calmette-Guérin vaccination stimulated NK T cells in vivo to produce IL-21, which subsequently suppressed IgE, although allergic symptoms of the bacillus Calmette-Guérin-vaccinated animals were not documented. However, it remains obscure whether apoptosis and/or Bmf expression was induced in B ϵ cells in vivo. Our present findings strongly suggest that Id2-dependent suppression of IgE CSR may predominantly participate in the IL-21-mediated suppression of IgE in allergic animals, whereas apoptosis of B ϵ cells could also be induced in vivo by this pleiotropic cytokine.

In conclusion, we demonstrated that i.p. administration of IL-21 totally arrested the systemic anaphylaxis induced in a peanut allergy model that was otherwise extremely severe. Finally, it is strongly suggested that IL-21 may provide a promising clinical tool to control IgE-based allergic diseases including anaphylaxis.

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Disclosures

The authors have no financial conflict of interest.

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