

Expression and release of IL-29 by mast cells and modulation of mast cell behavior by IL-29

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Abstract

Background: The role of interleukin (IL)-29 in innate immunity has been recognized recently, and it is regarded as a potent bioactive molecule. However, little is known about its role in the pathogenesis of allergy. Because mast cells are recognized as primary effector cells of allergy, we investigated the potential relationship between IL-29 and mast cells in this study.

Objective: To examine the expression of IL-29 in mast cells and the influence of IL-29 on mast cell mediator release and accumulation.

Methods: Expression of IL-29 in mast cells was determined by double-labeling immunohistochemistry and flow cytometry analysis. Mast cell cell-line was cultured to examine the mediator release, and mouse peritoneal model was employed to observe the mast cell accumulation.

Results: Large proportions of mast cells expressing IL-29 were localized in human tissue including the colon, tonsil and lung. Mast cells can release substantial quantity of IL-29 upon challenge with proteolytic allergens. Extrinsic IL-29 provoked IL-4 and IL-13 release from mast cell line P815 cells through PI3K/Akt and (JAK)/STAT3 signaling pathways, but failed to induce mast cell histamine release from human mast cells. Extrinsic IL-29 also induced mast cell infiltration in mouse peritoneum by a CD18- and ICAM1-dependent mechanism.

Conclusion: Mast cell-derived IL-29 has the potential to be involved in the pathogenesis of allergic inflammation.

Interferon (IFN)- λ , a newly identified molecule, has three subtypes, IFN- λ 1 (IL-29), IFN- λ 2 (IL-28A) and IFN- λ 3 (IL-28B), which belongs to the type III IFN family (1) as well as is categorized to the superfamily of human class II cytokines (2). IFN- λ family shares with type I IFN the same pathway driving expression of a common set of genes. IFN- λ binds to a distinct membrane receptor, composed of IFNLR1 and IL10R2.

IFN- λ exhibits several common features with type I IFNs: antiviral activity, antiproliferative activity and *in vivo* antitu-

mour activity. Although IFN- λ is only recently identified (1), its immune regulatory activities have attracted high attention (3). It was first identified that IFN- λ was induced upon viral infection. Because its expression suppresses the intracellular viral infection, the function of IFN- λ is proposed as antiviral initially (3). Subsequent studies found that the function of IFN- λ is not limited to antiviral, is also involved in immune regulatory activities, such as the regulation of T helper (Th)1/Th2 responses (4), modulation of dendritic cell function and alteration of regulatory T cell proliferation (5). However, whether IFN- λ is involved in the pathogenesis of allergic disorders is unclear.

Mast cells are the primary effector cells in allergic reactions (6). They release an array of chemical mediators, such

Abbreviations

mAb, monoclonal antibody; PMB, polymyxin B; rhIL-29, recombinant human IL-29; rPer, recombinant Per a 1.0102; Th, T helper.

as histamine, tryptase, to induce allergic clinical symptoms. A number of mast cells accumulate on the sites of allergic responses, such as in the nasal mucosa, lower airway (7), skin (8) and gastrointestinal tract (9). Mast cell-deficient mice do not develop allergic responses. In fact, mast cell accumulation founds the basis of allergic attacks (10). However, factors responsible for mast cell accumulation in allergic sites are not fully understood.

Here, we report a novel phenomenon on mast cell biological behavior that has not been described previously. Mast cells expressed IL-29. Mast cells released IL-29 in response to stimulation of cockroach allergen. Furthermore, extrinsic IL-29 induced mast cells to release Th2 cytokines and attracted mast cells to the peritoneal cavity.

Materials and methods

Human specimens and animals

Specimens for immunohistochemistry, flow cytometry analysis and mast cell challenge were collected from the Department of Pathology, Shantou University Medical College. Macroscopically normal lung or colon tissues were removed at lobectomy or enterectomy from patients with carcinoma, respectively, and tonsillar tissue was removed at tonsillectomy. The protocol for ethical use of human tissue in research was approved by the Ethical Committee of the Medical College, Shantou University.

BALB/c mice (18–22 g) were obtained from Guangdong Experimental Animal Centre, China, Grade II, Certificate No 2001A049. The animal experiment procedures were approved by the Animal Care Committee at Shantou University.

Cell lines and culture

Mouse mast cell line P815 cells and RBL-2H3 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured with ATCC complete growth medium or Dulbecco's Modified Eagle Medium (DMEM). Human mast cell line, HMC-1, cells was a present from Dr. Joseph H. Butterfield (Mayo Clinic, MN, USA). Cells were cultured in 75-cm² tissue culture flasks (Falcon; VWR International, Mississauga, Canada) at 37°C in a 5% (v/v) CO₂, water-saturated atmosphere.

Double-labeling immunohistochemistry

Specimens fixed in Carnoy's fixative and embedded in paraffin wax. The staining procedure was mainly adopted from the procedure we described previously (11). For each section, the number of positively stained cells was counted in at least 30 fields (the area of each field equals 0.19 mm²). For each tissue type (colon, lung and tonsil), six different donors were examined.

Flow cytometry analysis

Nucleated cells were isolated from the surgically removed human tissues with the procedures reported previously (12).

After fixed in 70% ethanol, cells were stained with fluorescence-labeled antibodies for 1 h on ice. The stained cells were analyzed by flow cytometry (FACScalibur; BD Bioscience, San Jose, CA, USA). Data were processed with CELLQUEST software.

Challenge of HMC-1 cells

Cells were exposed to various concentrations of histamine, rPer, LPS and poly (I:C) with or without their inhibitors, respectively. At 2, 6 or 16 h following incubation, culture supernatant in each well was collected.

Mast cell challenge and analysis of histamine release

The procedure was mainly adopted from the procedure described previously by He et al. (13). Purified recombinant human IL-29 (rhIL-29) at various concentrations or control secretagogues were incubated with mast cells for 15 min at 37°C. For priming experiments, cells were preincubated with rhIL-29 for 30 min before adding secretagogues.

A glass fiber-based, fluorometric assay was employed to determine histamine level in supernatants. Histamine release was expressed as a percentage of total cellular histamine levels, and corrected for the spontaneous release measured in tubes in which cells had been incubated with the HBSS diluent alone.

Challenge of P815 cells and analysis of signal transduction pathways

The procedure was mainly adopted from the procedure described previously by Zhang et al. (14). Cells were exposed to various concentrations of rhIL-29 or IFN- γ with or without its blocking antibody for 2, 6 or 16 h, respectively. For cell signaling experiments, the inhibitors of cell signaling pathways including PD98059, U0126, U0124, SB203580, LY294002 and AG490 were incubated with cells for 30 min before being challenged with rhIL-29 for 15 min, 2 h or 6 h. The phosphorylation of Akt, ERK, p38 and STAT3 was analyzed by using Cellular Activation of Signaling ELISA (CASE) kits as described previously by Zhang et al. (14).

ELISA

Levels of IL-4, IL-6, IL-10, IL-12, IL-13 and histamine in culture supernatant of P815 cells and IL-29 in culture supernatant of HMC-1 cells were measured by using ELISA kits according to the manufacturer's instruction.

Mouse peritoneal mast cell accumulation test

The procedure was mainly adopted from the procedure described previously by He et al. (15). Briefly, rhIL-29, LPS, RANTES, IL-8, bradykinin or human serum albumin were injected into the peritoneum of mice for 3, 6 or 16 h. After collecting peritoneal lavage, cytospin preparations were made, and cells were stained with modified Wright's stain. Differential cell counts were performed for a minimum of

500 cells. The results were expressed as absolute number of mast cells per mouse peritoneum.

For the experiments investigating mast cell migration mechanism, groups of mice were pretreated intravenously with mAbs against L-selectin, CD11a/CD18 and ICAM-1; sodium cromoglycate or terfenadine respectively for 30 min before intra-peritoneal injection of rhIL-29. At 6 h following injection, the mice were killed, and their peritoneal lavages were processed as described earlier.

Statistics

The values were expressed as the means $\pm$ SD of at least three independent experiments. The data were analyzed using the two-tailed unpaired Student's *t*-test when data were consisted of two groups or by ANOVA when three or more groups were compared. $P < 0.05$ was accepted as statistically significant.

Results

Mast cells express IL-29

Because mast cells are the major effector cells in allergic reactions, and they express INF- γ (16) and IL-10 (17), we

hypothesized that mast cells may express IL-29. To this end, biopsy specimens from the lung, colon and tonsil were examined for IL-29⁺ mast cells with immunohistochemistry approach. After stained with antibodies against IL-29 and tryptase, we found that up to 60% mast cells were IL-29 positive (Figs 1 and S1). To further confirm the expression of IL-29 by mast cells, we isolated nucleated cells from surgical removed tissue and stained with antibodies against IL-29 and tryptase. As analyzed by flow cytometry data, although only a small portion of cells were IL-29 positive, with gating technique, over 60% IL-29⁺ cells are also tryptase positive (Fig. 2).

Regulation of IL-29 release from human mast cells

Because IL-29 was localized in mast cells, we wondered whether the IL-29 could be released. Thus, our next attempt was to observe the IL-29 release from mast cells by treating mast cell cell-line with several bioactive molecules. We first tested the capacity of a common allergen, cockroach-derived Per a 7 that can activate mast cells to release cytokine IL-4 and IL-13 from mast cells (18, 19). As expected, the levels of IL-29 in HMC-1 cell culture supernatant were elevated up to 38% at 16 h (not 2 and 6 h) following addition of rPer

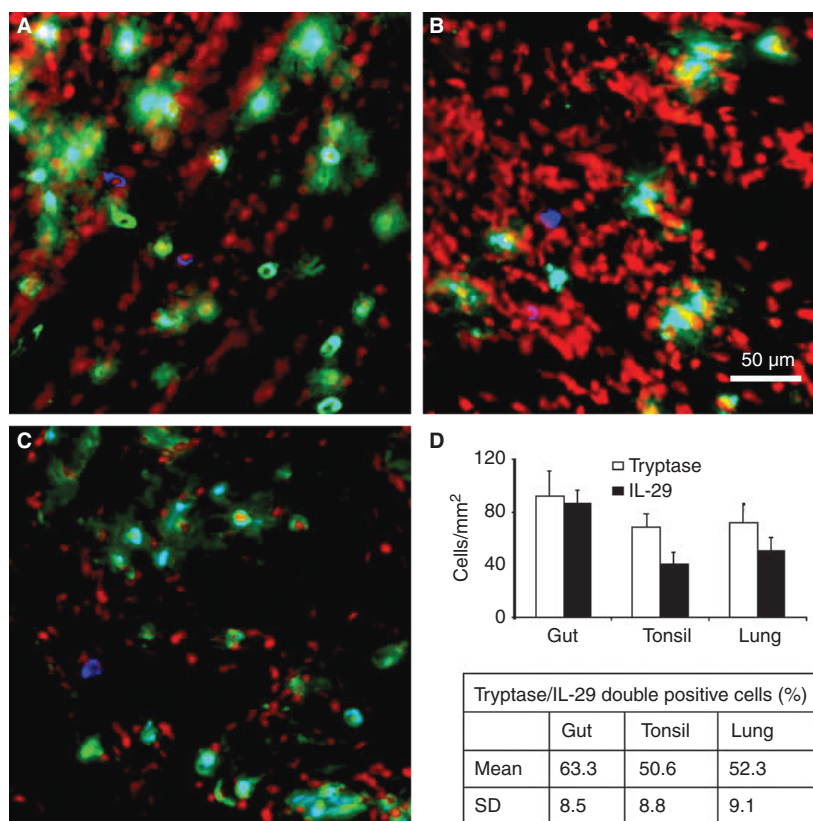


Figure 1 Expression of IL-29 in human mast cells. A–C, confocal images indicate the expression of IL-29 (in blue) in mast cells (in green) in surgically removed tissue of the colon (A), tonsil (B) and lung (C). Panel D shows the cell counts in sections (30 fields were

counted for each sample). Data were expressed as mean $\pm$ SD. Isotype IgG stained sections did not show any positive staining (data not shown).

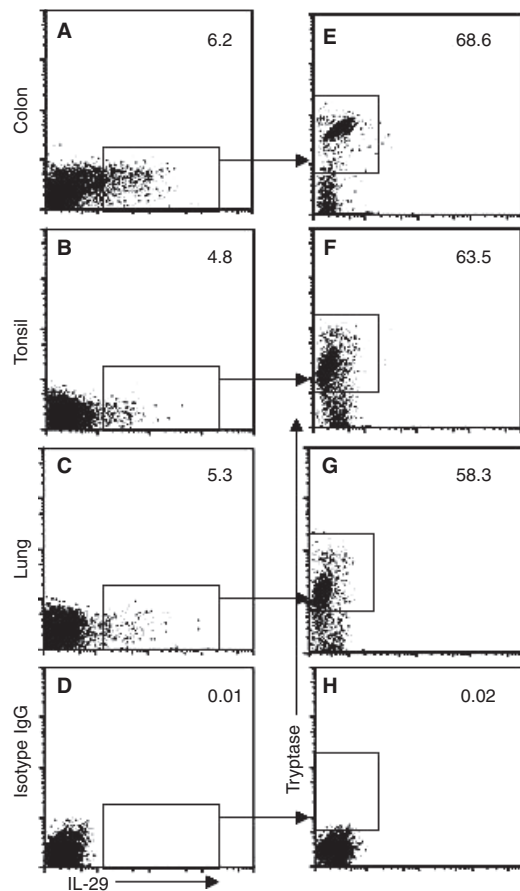


Figure 2 Flow cytometry shows IL-29 expression in human mast cells. Human tissue was obtained as described in Figure 1. Single cells were isolated and processed for flow cytometry. A–D, dot plots indicate IL-29⁺ cell population (the gated cells) in isolated cells. E–H, with gating technique, the gated cell population in panel A–D were also trypsinase positive (the gated cells).

(Fig. 3A), which fell back to control levels at 24 h after (data not shown). The action of rPer on IL-29 expression in HMC-1 cells was abolished by addition of blocking antibody PA1 to the culture (Fig. 3A). We also examined the effect of lipopolysaccharide (LPS) and poly (I:C) on the expression of IL-29 in mast cells. LPS induced a dose-dependent upregulation of expression of IL-29 (up to 40%) in HMC-1 cells following 6- and 16-h incubation period that could be reduced to 79.7% by Polymyxin B (PMB, a neutralizer of LPS) (Fig. 3A). Poly (I:C) at 25 µg/ml elicited small but nevertheless significant upregulation of IL-29 expression at 6 h (not 2 and 16 h) following incubation. An anti-human TLR3 antibody was able to block approximately 78.2% poly (I:C) action (Fig. 3A).

rPer induced also concentration-dependent release of IL-29 from HMC-1 cells following 16-h incubation period. Approximately up to 2.0-fold increase in IL-29 release was achieved when rPer was incubated with cells (Fig. 3B). Similarly, approximately up to 2.2-fold increase in IL-29 release from

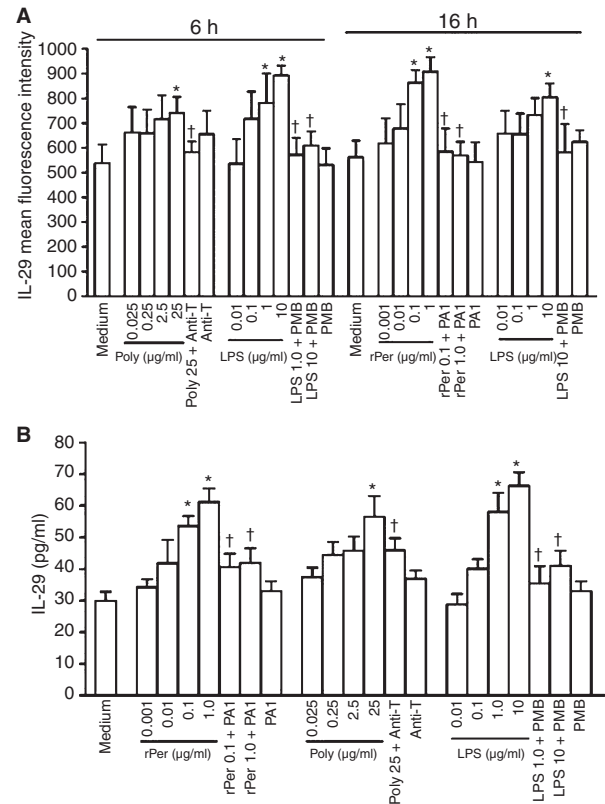


Figure 3 Allergens induce expression of IL-29 in human mast cells. Human mast cell line (HMC-1) cells were exposed to cockroach allergen, Per, for 16 h in culture. The levels of IL-29 were detected by flow cytometry (A) and ELISA in culture media (B). Bars indicate the IL-29 levels (presented by the median fluorescence intensity) within gated trypsinase⁺ cells (A) and culture media (B). Values are presented as the means $\pm$ SE from four separate experiments. * P < 0.05, compared with the medium alone control. † P < 0.05, compared with those were not treated with inhibitors. Poly: poly (I:C). Anti-T: anti-TLR3 antibody (20 µg/ml). PMB: blocker of LPS (20 µg/ml). PA1: Anti-rPer antibody (20 µg/ml).

HMC-1 cells was observed when LPS was incubated with HMC-1 cells for 16 h (Fig. 3B). At a concentration of 25 µg/ml, poly (I:C) induced 1.9-fold increase in IL-29 release. PA1, PMB and Anti-T (all at 20 µg/ml) was able to block rPer, LPS and poly (I:C) induced IL-29 release by 61.5, 67.2 and 40%, respectively (Fig. 3B). rPer, LPS and poly (I:C) at the concentrations examined had little effect on IL-29 release from HMC-1 cells at 2 and 6 h following incubation (data not shown).

Extrinsic IL-29 induces Th2 cytokine release from mast cells

As aforementioned (Fig. 3), IL-29 could be released to culture media. The fact indicates that IL-29 can be released to the micro milieu from mast cells. Therefore, it may affect cytokine production of mast cells. We chose P815 cells, a mouse mast cell line which does not express IL-29, as

an experiment model to examine the influence of extrinsic IL-29 on cytokine release from mast cells. P815 cells were exposed to recombinant human IL-29 at concentrations from 0.01 to 100 ng/ml in culture. As shown by ELISA, levels of IL-4 and IL-13, but not IL-10 or IL-12 were increased in culture media following 6- and 16-h incubation. Approximately up to 2.8- and 2.9-fold increases in IL-4 and IL-13 in culture media were achieved, respectively, at 6-h time point (Fig. 4A, B). rhIL-29 appeared more potent than IFN- γ , but much less potent than calcium ionophore in induction of release of IL-4 and IL-13 (Fig. 4A, B). Exposure to rhIL-29 also increased IL-6 up to 2.0-fold in culture media following 16-h incubation (Fig. 4C). We also observed the cytokine release from p815 cells fell back to control levels at 24 h after addition of IL-29 (data not shown). Treatment with SC2 antibody completely abolished

rhIL-29 elicited IL-4, IL-13 and IL-6 release from P815 cells (Fig. 4).

Extrinsic IL-29 does not cause histamine release from human mast cells

Activation of mast cells by cytokines has been demonstrated previously. Stem cell factor (20) and TNF- α (21) were reported to provoke mast cells to release mediators. To determine whether rhIL-29-induced cytokine release resulted from mast cell degranulation, we performed separate experiments to examine the effects of rhIL-29 on human mast cells. As shown by a glass fiber-based, fluorometric assay, no detectable increase in histamine (a marker of mast cell degranulation) levels in culture supernatant was noted at IL-29 concentrations of 1, 10 and 100 ng/ml. Alternatively, with the same approaches ($n = 5$ different donors for each tissue type examined), 10 μ g/ml of anti-IgE antibody and 1 μ g/ml of calcium ionophore provoked $12.3 \pm 2.0\%$ and $32.1 \pm 6.9\%$, $12.9 \pm 1.8\%$ and $36.2 \pm 8.2\%$ or $22.5 \pm 4.6\%$ and $30.9 \pm 6.7\%$ net histamine release from lung, colon or tonsil mast cells, respectively. Collectively, IL-29 does not induce mast cell degranulation, nor prevent other secretagogues from activating mast cells.

Inhibition of rhIL-29-induced IL-4 and IL-13 release and phosphorylation of Akt and STAT3 by signaling inhibitors

Elements of mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/Akt signal transducer and activator of transcription (STAT) 1 and 3 are involved in the signal transduction pathway by which IL-29 binds to their receptor complex to initiate protein expression (22). We inferred that the IL-29-involved biological activities could be abolished by blocking these signal transduction pathways. With the P815 cell culture model, we treated cells with Akt antagonist LY294002, or STAT3 inhibitor AG490 30 min prior to exposure to extrinsic IL-29. As expected, LY294002 (20 μ M) completely abolished rhIL-29-induced phosphorylation of Akt in P815 cells (Table 1); AG490 (40 μ M) abolished rhIL-29-induced phosphorylation of STAT3 by rhIL-29 (Table 1). LY294002 also inhibited rhIL-29-induced IL-4 and IL-13 release from P815 cells by up to 91.2% and 73%, respectively, whereas AG490 inhibited rhIL-29 provoked IL-4 release by 87.9% when they were preincubated with the cells for 30 min. In contrast, PD98059, a MAPK pathway inhibitor at 50 μ M, U0126, an inhibitor of ERK at 5 μ M, U0124, a structural analog negative control of U0126 at 5 μ M and SB203580, a selective inhibitor of p38 MAPK at 20 μ M had little influence on rhIL-29-induced cytokine release (data not shown).

Effect of extrinsic IL-29 on mast cell accumulation in mouse peritoneum

It is still an enigma what induces mast cell infiltration. Because IL-29 has the ability to modulate mast cell's activities as aforementioned, we speculated that IL-29 has the capability to

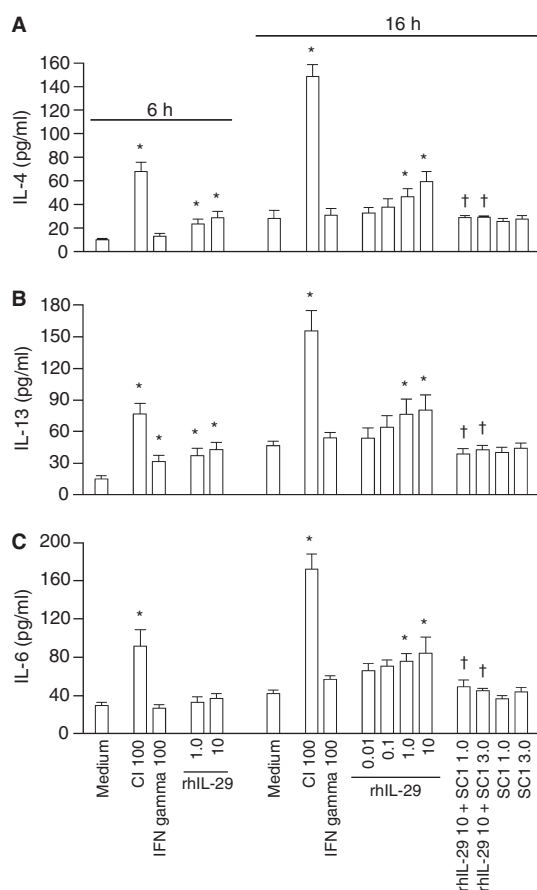


Figure 4 Extrinsic IL-29 induces release of Th2 cytokines. Mouse mast cell line P815 cells were treated with rhIL-29 in culture for 16 h. Levels of IL-4, IL-13 and IL-6 in culture media were determined by ELISA. Bars indicate the levels of IL-4 (A), IL-13 (B) and IL-6 (C) (mean $\pm$ SEM for four separate experiments). * $P < 0.05$, compared with medium control. † $P < 0.05$, compared with those were not treated with inhibitors. SC2 = monoclonal antibody against IL-29.

Table 1 Effect of LY294002 (LY, 20 μ M) and AG490 (AG, 40 μ M) on rhIL-29-induced phosphorylation of Akt and STAT3

Compound		15 min	120 min
		Phospho-Akt (OD450:OD595)/ pan(OD450:OD595)	
rhIL-29	10	0.23 $\pm$ 0.03	0.47 $\pm$ 0.07
	100	0.27 $\pm$ 0.07	0.59 $\pm$ 0.08
rhIL-29	10 + LY	0.1 $\pm$ 0.02*	0.13 $\pm$ 0.03*
	100 + LY	0.1 $\pm$ 0.01*	0.12 $\pm$ 0.03*
		Phospho-STAT3 (OD450:OD595)/ pan(OD450:OD595)	
rhIL-29	10	0.68 $\pm$ 0.11	0.85 $\pm$ 0.09
	100	0.77 $\pm$ 0.17	0.97 $\pm$ 0.15
rhIL-29	10 + AG	0.60 $\pm$ 0.08	0.47 $\pm$ 0.06*
	100 + AG	0.67 $\pm$ 0.11	0.55 $\pm$ 0.07*

Values shown are mean $\pm$ SEM for four separate experiments. P815 cells were treated with LY or AG for 30 min before being challenged with rhIL-29 (ng/ml) for 15 min or 2 h. Phosphorylation of Akt and STAT3 was determined by using signaling ELISA analysis. * P < 0.05 in comparison with the uninhibited control. rhIL-29, recombinant human IL-29.

induce mast cell infiltration. Mouse peritoneum was used as a study platform as mouse does not produce functional IL-29 by itself (23). The results showed that at concentration of 1.0, 10 and 100 ng/ml, rhIL-29 peritoneal injection (ip) induced mast cell accumulation in mouse peritoneum in a concentration-dependent manner. Up to approximately 2.9 (range 1.3–6.6) -fold increase in the number of mast cells was observed in mouse peritoneum 6 h following injection with IL-29 (Fig. 5A). As a comparison, in separate experiments, administration (ip) of RANTES and bradykinin (both at 100 ng/ml) induced 3.3- (range 1.2–9.8) and 3.4 (range 1.1–12.1)-fold increases in the number of mast cells in peritoneal cavity, respectively. Time course study showed that the mast cell accumulation in peritoneal cavity was initiated at 3 h after rhIL-29 deliver (ip), peaked at 6 h and declined at 16 h following IL-29 injection (Fig. 5A). Notably, rhIL-29-induced mast cell accumulation was inhibited by SC2 antibody up to 79.8% (Fig. 5A). Intravenous injection of mAbs against CD18, CD11a and ICAM-1 30 min prior to intra-peritoneal injection of rhIL-29 blocked rhIL-29-induced mast cell accumulation by up to 91.8%, 95.7% and 94.1%, respectively (Fig. 5B). However, treatment with anti-L-selectin, isotype IgG (Fig. 5 B), terfenadine and sodium cromoglycate had little effect on the rhIL-29-induced mast cell infiltration (data not shown).

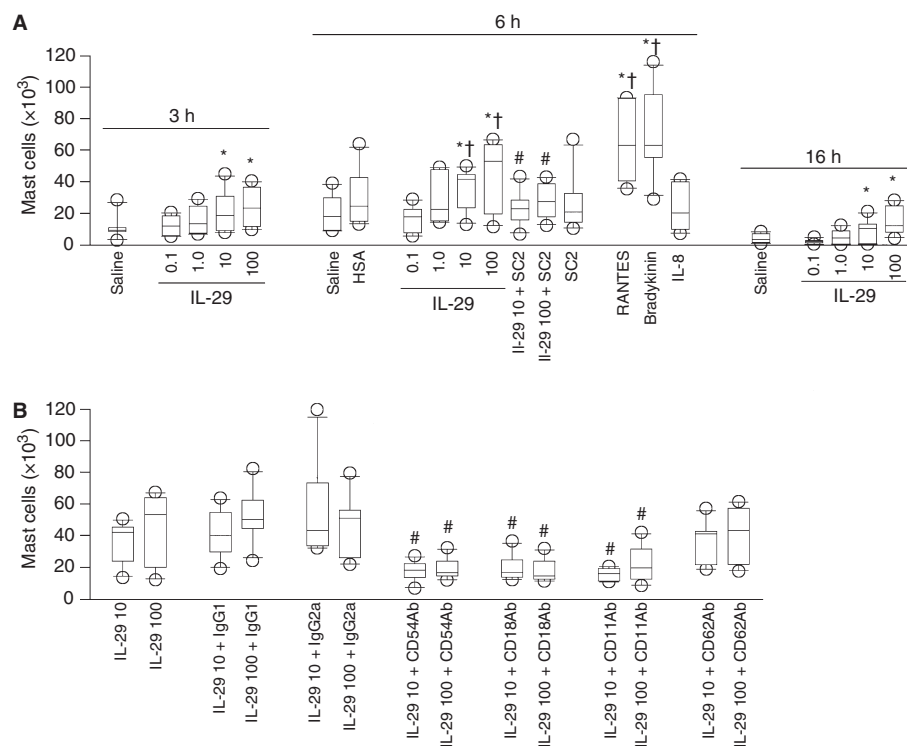


Figure 5 IL-29 induces mast cell accumulation in the peritoneal cavity. Recombinant human IL-29 were injected into the peritoneum of mice. The peritoneal lavage fluid was collected at 3, 6 or 16 h afterward. Data were displayed as a boxplot, which indicates the median, interquartile range, the largest and smallest values other than outliers (whiskers) and the outliers (○). Each data represented a group of the absolute numbers of mast cells in

500 counted nucleated cells isolated from collected peritoneal lavage fluid for 6–7 animals. * P < 0.05, compared with saline control. † P < 0.05, compared with HSA control. # P < 0.05, compared with those were not treated with inhibitors. SC2 = monoclonal antibody specific for IL-29 at 1 μ g/ml; IgG1 = hamster IgG1 isotype standard and IgG2a = rat IgG2a isotype standard.

Discussion

As the major effector cell type in allergic disorders, mast cells express numerous cytokines such as IL-4, IL-13 and IL-10. Using SC1, a mAb against human IL-29 raised in this study, and double-labeling immunohistochemistry and flow cytometry techniques, we demonstrate for the first time that large proportions of MC_T and MC_{TC} subtypes of mast cells in human lung, tonsil and colon tissues contain IL-29. Because the tissue specimen used in this study were from macroscopically normal tissues (except tonsil tissue), we anticipate that IL-29 can be stored in inactivated mast cells in the body. We also found that mast cells can release IL-29 in response to LPS and poly (I:C) and allergen, which indicates strongly that IL-29 is likely to contribute to innate as well as acquired immunity in the body.

Exposure to IL-29 induces mast cells to release Th2 cytokines such as IL-4 and IL-13, but not histamine, which indicates that IL-29 does not trigger the activation of mast cells, but selectively secrete certain cytokines. Our recent study shows that mast cell can respond to microbial stimulation and release IL-4 that can be regarded as one of the sources of early IL-4 (24). The 'early IL-4' is a critical molecule in the initiation of Th2 response (25). Exposure to IL-29 also drive mast cells to release IL-4, which further strengthen the on-going Th2 inflammation or/and initiate new antigen specific Th2 inflammation (24).

IL-29 has been reported to be able to activate STAT3 in tumor cell lines (26) and Akt in intestinal epithelial cell lines (22). These are also important components in signal transduction pathways of Th2 responses (19, 27). Indeed, PI3K/Akt signaling pathway and JAK/STAT3 signaling pathway appear to be responsible for the downstream signal transduction of IL-29-induced IL-4 release in P815 cells, whereas IL-29-induced IL-13 release seems through PI3K/Akt signaling pathway.

It is well known that mastocytosis is one of the major anatomical pathological features in allergic disorders. However, little is understood regarding how mast cells infiltrate into local tissue under an allergic environment. Here, we have provided the first evidence that IL-29, a newly identified cytokine, is able to induce mast cell infiltration *in vivo*. The time course study showed that IL-29-induced mast cell accumulation is a relatively rapid process, suggesting a novel self-amplification mechanism of mast cell accumulation, which mast cell releases IL-29 upon stimulation and then released IL-29 recruits more mast cells to the involved site. Induction of mast cell accumulation by cytokines such as stromal cell-derived factor-1 (SDF-1) (28), SCF (29) and IL-4 (30) has been found before, which may support our previous observation. In the parallel experiments, RANTES and bradykinin also showed their ability in induction of mast cell accumulation. Taking these findings together, we believe that there must be a self-amplification mechanism of mast cell accumulation in the body.

Because adherence and migration mechanisms of mast cells still remain obscure, it is difficult for us to draw a clear picture of the mechanisms, through which IL-29 induces mast

cell accumulation. However, CD18 and its heterodimer binding chain CD11a, and ICAM-1 (a ligand of CD18) (31) appear to be involved in IL-29-induced mast cell accumulation. ICAM-1 has been reported to be involved in IL-4-induced homotypic aggregation of mast cells (32), and it can act cooperatively with L-selectin to mediate mast cell accumulation (33). Therefore, selective induction of IL-4 release by rhIL-29 may be partially responsible for IL-29-induced mast cell accumulation.

In conclusion, mast cells can be actively involved in the innate immunity and the pathogenesis of allergic inflammation through release of IL-29. Our hypothesis is that viral or bacteria infection stimulates inflammatory cells to secrete IL-29, and released IL-29 on the one hand protects the body against viral infection, and on the other hand elicits mast cells to secrete IL-4 and IL-13 and recruits mast cells. These Th2 cytokines then trigger the process of allergic inflammation, such as intrinsic asthma, and accumulated mast cells exacerbate the asthma attack by releasing their inflammatory mediators. Induction of IL-29 release from mast cells by allergen without apparently causing mast cell activation indicates that mast cell cytokine release and degranulation may be via different mechanisms, which may help to explain the reasons why certain mast cell stabilizers such as sodium cromoglycate can inhibit mast cell degranulation *in vitro*, but does not effective in treating allergic diseases including asthma.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Split images of Fig.1. IL-29 was stained in blue. Tryptase was stained in green. The nuclei were stained with propidium iodide for morphological view.

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Supplements

Expression and Release of IL-29 by Mast Cells and Modulation of Mast Cell Behavior by IL-29

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Reagents

Polyinosinic-polycytidylic acid [poly (I:C)], lipopolysacchanides (LPS), polymyxin B, SB203580 (Sigma Aldrich, St. Louis, MO, USA), ECL kit, mouse IL-4, IL-12 and IL-10 ELISA kits (Rockford, IL, USA). Histamine kit (Copenhagen, Denmark). Antibodies against tryptase (FL-275) and IL-29 (B21) (Santa Cruz, Santa Cruz, CA, USA). Histamine ELISA kits were from Immunotech Co. (Marseille, France). Human IL-29 ELISA kit and mouse anti human toll like receptor (TLR)3 antibody (eBioScience, Los Angeles, CA, USA). PD98059, U0126, AG490, methylthio, U0124 and LY294002 (Cell Signaling Technology, Beverly, MA, USA). Cellular activation of signaling ELISA CASE™ kits for serine/threonine protein kinase (Akt), extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (MAPK) and signal transducer and activators of transcription 3 (STAT3) (SuperArray Bioscience Corporation, Frederick, MD, USA). Recombinant human IL-29 was prepared by us as described previously (34).

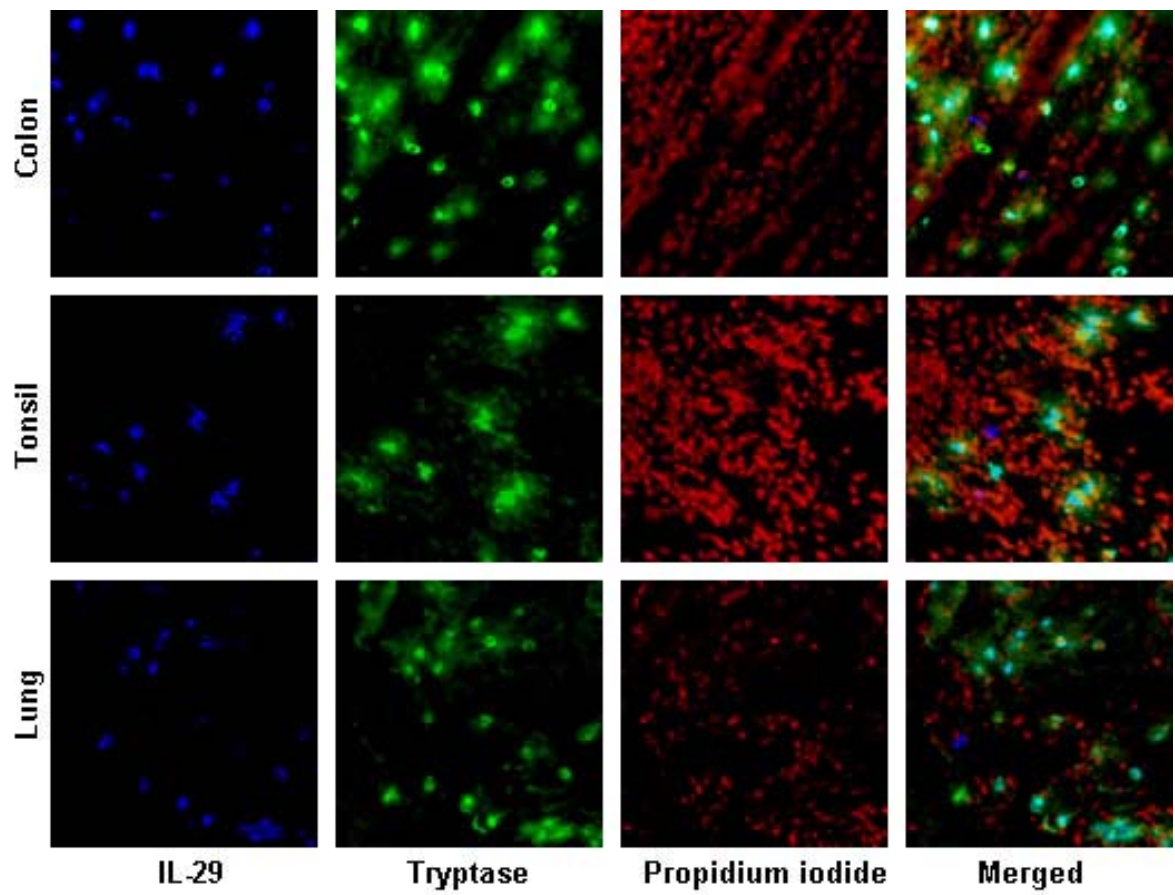


Figure S1. Split images of Figure 1. IL-29 was stained in blue. Tryptase was stained in green. The nuclei were stained with propidium iodide for morphological view.