Cytokine 117 (2019) 84–90

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

The role of IL-36γ and its regulation in eosinophilic inflammation in allergic rhinitis

Xiaowei Qin, Mengting Liu, Shuoying Zhang, Chunrui Wang, Tianhong Zhang*

Department of Otolaryngology, First Affiliated Hospital to Harbin Medical University, Harbin, China

ARTICLE INFO

Keywords:
Interleukin-36
IL-36R
Eosinophil
Human nasal epithelial cells
Allergic rhinitis

ABSTRACT

Background: Allergic rhinitis (AR) is characterized by eosinophilic inflammation. However, the function and regulation of eosinophils in AR are largely unknown. This study aimed to explore the expression and role of interleukin-36 (IL-36) cytokines in AR.

Methods: Sixty AR patients and 20 control subjects were recruited in this study. The mRNA and protein expression of serum IL-36 family cytokines and IL-36R in AR were detected by quantitative RT-PCR and enzyme-linked immunosorbent assay ELISA, respectively. IL-36R expression and regulation by eosinophils and the role of IL-36γ in the survival, adhesion, migration and activation of eosinophils were performed in purified eosinophils. Human nasal epithelial cell line was cultured and treated with different stimulators and IL-36γ was measured.

Results: The mRNA and protein expression of serum IL-36 cytokines and IL-36R were significantly higher in AR compared with control, especially in asthmatic patients. Among the IL-36 cytokines, the expression of IL-36γ was the highest. The expression of IL-36R by eosinophils were significantly increased compared with normal controls and was up-regulated by recombinant IL-17, IL-25, IL-33 and *Dermatophagoides pteronyssinus* group 1. The IL-36γ promote the survival, adhesion, migration and activation of eosinophils. Human nasal epithelial cells can secrete IL-36γ after treated with recombinant IL-17, IL-25, IL-33.

Conclusions: High expression of IL-36γ exaggerates eosinophilic inflammation in AR by promoting the survival, adhesion, and activation of eosinophils.

1. Introduction

Allergic rhinitis (AR), which is characterized by eosinophilic inflammation of upper airway, often presented as enhanced Th2 immune response, migration of eosinophils into the airway and airway mucus production. Therefore, eosinophils are crucial effector cells in the allergic inflammatory reactions. After stimulation, eosinophils release cytotoxic granule proteins, reactive oxygen species and lipid mediators, which caused tissue injury, vascular leakage, and mucus secretion [1].

IL-36 cytokine family members, which includes IL-36α, IL-36β, IL-36γ, and IL-36 receptor antagonist (IL-36Ra), are grouped into the IL-1 family [2]. IL-36 receptor complex composed of IL-36R and IL-1R accessory protein (IL-1RAcP) as a co-receptor [3]. IL-36 cytokines can be induced by monocytes/macrophages, T cells, neuron cells, keratinocytes, and bronchial epithelial cells [4–8]. Correspondently, IL-36R is expressed by skin keratinocytes, fibroblasts, glial cells, and various immune cells [3,9–11]. IL-36α, IL-36β, IL-36γ act as agonists for IL-36R, whereas IL-36Ra and IL-38 function as antagonists for IL-36R signaling by competitively binding to IL-36R [12–15]. Recently, IL-36 cytokines were proved to be engaged in neutrophil infiltration in psoriasis [16]. Moreover, increased production and activation of IL-36γ may act on neutrophils and further exaggerate neutrophilic inflammation in chronic rhinosinusitis [17]. However, whether they play a role in eosinophilic inflammation in AR is not known.

In the current study, we aimed to explore the role of IL-36 in AR and its regulation in eosinophilic inflammation in AR.

2. Methods

2.1. Patients

Sixty AR patients (18–60 years old) and 20 control subjects were enrolled in this study (Table 1). Our study was approved by the First Affiliated Hospital of Harbin Medical University ethics committee and the parent's written informed consent. The diagnosis of AR were made according to Allergic Rhinitis and its Impact on Asthma (ARIA) guideline (2010) [18]. The patient's disease history, nasal symptoms (sneezing, rhinorrhea, itchy nose, and nasal congestion), and a positive

* Corresponding author at: No. 23, Youzheng Street, Harbin 150001, China.
E-mail address: crystalwang_18@163.com (T. Zhang).

https://doi.org/10.1016/j.cyto.2019.02.008
Received 27 September 2018; Received in revised form 17 January 2019; Accepted 7 February 2019
Available online 01 March 2019

Table 1
Demographic characteristic of AR and control patients.

Groups	AR group	AR with Asthma group	Control
Number	45	15	20
Sex (Male:Female)	23:22	7:8	11:9
Age (years)	35.3(23–46)	28.1(21–38)	32.2(25–42)
Eosinophil[a] (count/mm^3)	245(90–1280)*	128(105–1750)*,**	45(6–165)
Neutrophil[a] (count/mm^3)	6200(2100–18700)	12500(7000–19900)*,**	7800(2200–11500)
ECP[a] (ng/ml)	23.1(6.0–156.0)*	64.7(22.5–183.0)*,**	12.8(8.1–123.0)
TIgE (IU/mL)	117.2(41.5–789.2)*	156.7(87.1–1123.4)*	22.6(7.1–67.8)
Sensitization			
D1 (n)	25	10	0
D2 (n)	30	15	0
Cockroach (n)	8	0	0
Cat (n)	7	0	0
Dog (n)	4	0	0
Others (n)	1	0	0
Nasal steroid	30(75%)	10(67%)	0
Nasal antihistamine	35(78%)	9(60%)	0
Oral antihistamine	20(44%)	11(73%)	0
inhaled corticosteroid	0	12(80%)	0

[a] Data presented as median values (minimum–maximum). D1, Dermatophagoïdes pteronyssinus. D2, Dermatophagoïdes farinae.
* Compared with control group, $P < 0.05$.
** Compared with AR group, $P < 0.05$.

Table 2
Primers for quantitative RT-PCR analysis.

Primer	Sequence
IL-36α	(F)5′-CAGCTGAAGGAAAAGGATATAATGGAT-3′ (R)5′-GCCACTCTGGCTGTGGTAGAA-3′
IL-36β	(F)5′-ACCAAGGAGAGAGGCATAACTAAT-3′ (R)5′-AGTGAACTCAGTCGCATAATGATC-3′
IL-36γ	(F)5′-TAGGACCTCCACCCTTGAGTC-3′ (R)5′-AATGATGGGCTGGTCTCTCTT-3′
IL-36Ra	(F)5′-AGGGAGGTGGTCATAGAGTCAG-3′ (R)5′-GAGGGAAGAGATAGGAAAGGTAGC-3′
IL-38	(F)5′-ACGCTTCACCTTCTTCCAGAG-3′ (R)5′-GCAGTTTCCTGTCTCCCTACC-3′
IL-36R	(F)5′-CTGGACAAGCCGTGGCCAATGT-3′ (R)5′-AGCCCAGCGATTCGGGGACC-3′
GUSB	(F)5′-GACACGCTAGAGCATGAGGG-3′ (R)5′-GGGTGAGTGTGTTGTTGATGG-3′

IL, interleukin; IL-36Ra, IL-36 receptor antagonist; GUSB, beta-glucuronidase.

atopic status were confirmed before AR was diagnosed. The atopic status was evaluated by a skin prick test or the detection of IgE (Phadia) specific to common inhalant allergens (dust mites, pollens, pets, molds, cockroach, etc.). Patients with other nasal disease (chronic rhinosinusitis, nasal tumor, etc.) were excluded from our study. For controls, 20 healthy volunteers were enrolled. All subjects were nonsmokers and free of respiratory tract infections and any drug usage history 4 weeks before the study.

2.2. Real-time PCR analysis

Total RNA was extracted from cells using TRIzol reagent (Life Technologies, Carlsbad, California) as described previously [19]. The cDNA was synthesized from 1 μg of total RNA using an oligo (dT) primer and M-MLV reverse transcriptase (TAKARA, Shiga, Japan). Quantitative PCR was performed by using an ABI PRISM 7300 Detection System (Applied Biosystems, Foster City, California). The sequences of the primers were listed in Table 2. Amplification was performed under 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10 s, and annealing extension at 60 °C for 60 s. Melting curve analysis was used to control for amplification specificity. Relative gene expression was calculated as mentioned in previous study [20]. Beta-glucuronidase (GUSB) was selected as a housekeeping gene for normalization of gene expression. The relative abundance of different IL-36 members in sinonasal mucosa was reflected by ΔCt values (ΔCt = the difference in threshold cycles for target and housekeeping gene GUSB). Therefore, high ΔCt value means low expression in target mRNA.

2.3. ELISA

The serum levels of IL-36α, IL-36β, IL-36γ, IL-36Ra, and IL-38 were detected in duplicate using ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. All experiments were repeated at least three times. The eosinophilic cationic protein (ECP) levels were measured by using the UniCAP system (Pharmacia, Uppsala, Sweden).

2.4. Preparation and culture of eosinophils

Eosinophils (15 mL) were prepared from atopic donors by MACS-negative immunomagnetic isolation kit (Miltenyi Biotec, Bergisch Galdbach, Germany) as described in the instructions. The eosinophils (> 98% purity and viability) were cultured in RPMI 1640 medium supplemented with 10% FBS (Gibco, New York, USA).

IL-4, IL-5, IL-6, IL-13, IL-17, IL-1β, IL-33 at 10 ng/mL (R&D systems, Minneapolis, MN, USA), IL-25 (R&D system) at 50 ng/mL, and *Dermatophagoides pteronyssinus* group 1 (Der p1) at 10 μg/mL (Prospec, East Brunswick, NJ, USA) were used for stimulation.

2.5. Functional experiments of eosinophil

Eosinophil apoptosis was performed using flow cytometric analysis (FACS Calibur, BD, New Jersey, USA). The percentage of viable eosinophils was determined by low mean fluorescence intensity (MFI) of both Annexin V-FITC and propidium iodide (PI)-PE.

For the adhesion assay, the eosinophil suspension was cultured in 96-well plates coated with 60 μl of fibronectin (R&D system) for 30 min at 37 °C (5% CO_2) with different stimulators. After incubation, the adhered cells were washed twice with PBS. The eosinophil adhesion was calculated by comparing the residual eosinophil peroxidase (EPO) activity of adherent cells to that of a standard curve as described previously [21].

For the migration assay, a 24-well Transwell system was used. In brief, the lower wells of the Boyden chamber were loaded with stimulators in RPMI medium and cell suspension of eosinophils was added to the upper wells of the chamber. After 6 h incubation at 37 °C (5% CO_2),

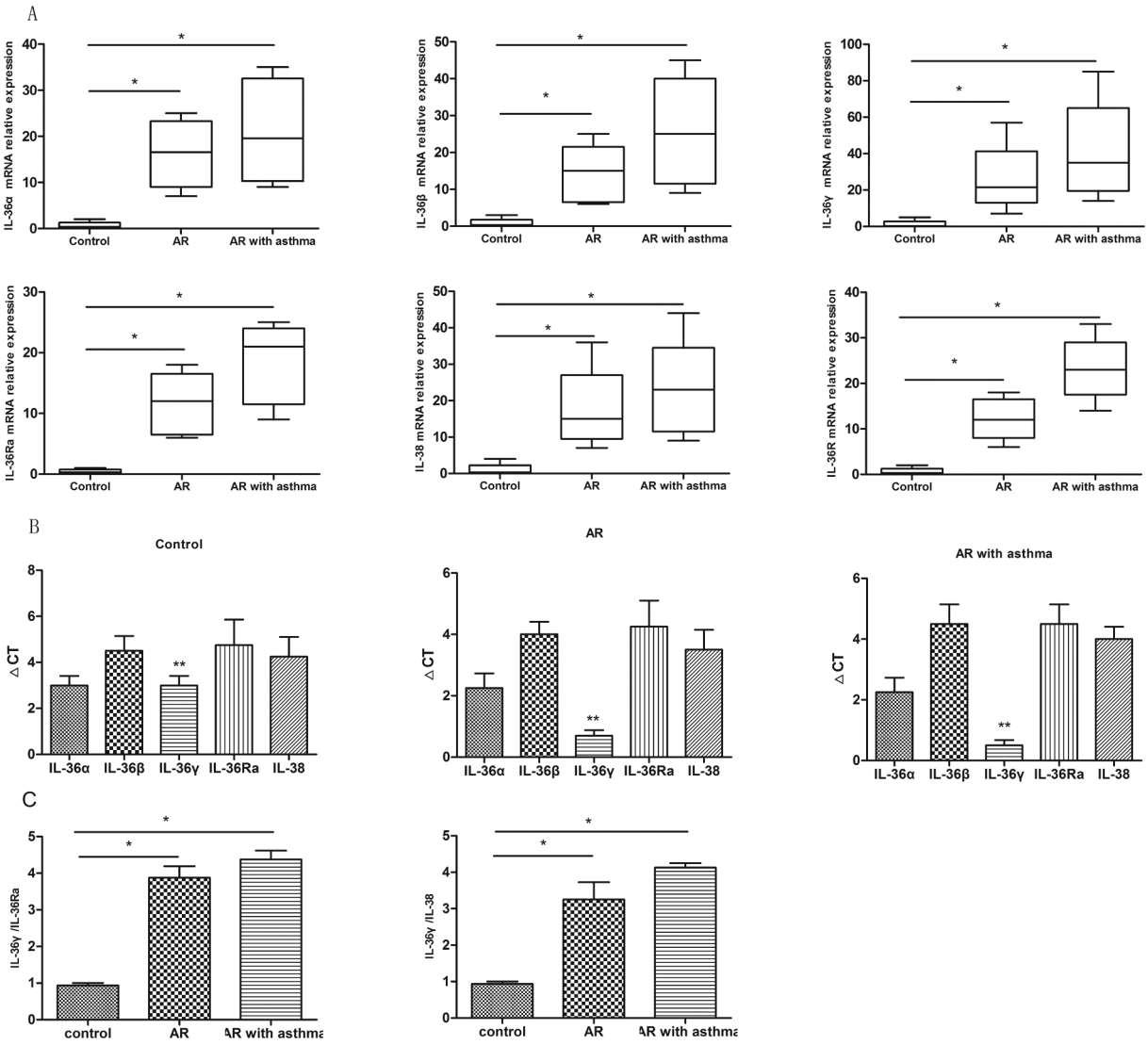

Fig. 1. The mRNA expression of IL-36 family cytokines and IL-36R in AR. A, The mRNA expression levels of IL-36 cytokines and IL-36R were elevated in AR and AR with asthma compared with controls. B, The relative mRNA expression abundance of IL-36 family members. C, The ratio of IL-36γ/IL-36Ra mRNA level and the ratio of IL-36γ/IL-38 mRNA level in different groups. $^{*}P < 0.05$, compared with the control group. $^{**}P < 0.05$, compared with other IL-36 family members.

the number of migrated cells was determined by Gimesa staining. Migrated cells were counted in 10 fields microscopically under 400× magnification. All the above results were from three independent experiments.

BAY11-7082 (1 μM, IκB-α phosphorylation inhibitor), U0126 (1 μM, MEK inhibitor), SP600125 (3 μM, Jun N-terminal kinase (JNK) inhibitor), SB203580 (7.5 μM, p38MAPK inhibitor), LY294002(5 μM, phosphatidylinositol 3-OH kinase (PI3K)/Akt inhibitor) and AG490 (10 μM, Janus kinase (JAK) inhibitor) were purchased from Calbiochem Corp.

2.6. Human nasal epithelial cell culture and treatment

Human nasal epithelial cell line (HNECs) was bought from American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in bronchial epithelial cell basal medium (BEBM, Lonza, Walkersville, MD, USA) supplemented with SingleQuot Kit Suppl (Lonza) at 37 °C in a 5% CO_2-humidified chamber. After the cells were differentiated, they were stimulated by various stimulators or inhibitors. After stimulation, cells were harvested for quantitative RT-PCR assay.

IL-4, IL-5, IL-6, IL-13, IL-17, IL-1β, and IL-33 at 10 ng/mL (R&D systems, Minneapolis, MN, USA), IL-25 (R&D system) at 50 ng/mL, poly (I:C) (dsRNA) at 25 μg/mL (Tocris, Bristol, UK), lipopolysaccharides (LPS) (InvivoGen, San Diego, CA, USA) at 1 μg/mL, staphylococcal enterotoxin B (SEB) (Millipore, Merck KGaA, Darmstadt, Germany) at 1 μg/mL, and Der p1 at 10 μg/mL (Prospec, East Brunswick, NJ, USA) used for stimulation. For nasal epithelial cells, SEB, LPS, dsRNA were used as mimics for bacterium and virus. We aimed to compared the IL-36γ expression by nasal epithelial cells under allergic (Der p1) or infection state (SEB, LPS, dsRNA).

2.7. Statistical analysis

All data are expressed as the medians and interquartile ranges. Statistical analysis was performed using the Kruskal-Wallis H test and the nonparametric Mann-Whitney *U* test except additional note. Correlations analysis were done by the Spearman rank correlation

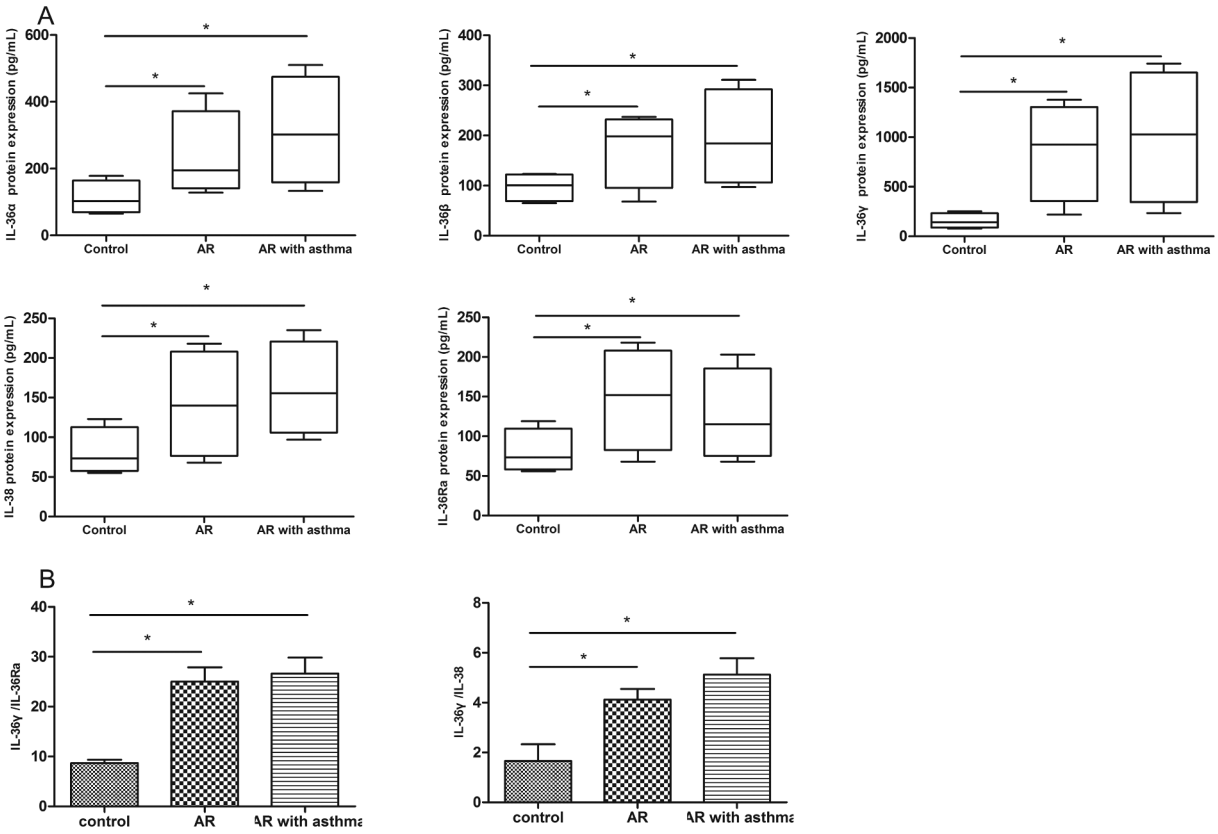

Fig. 2. The protein expression of IL-36 family cytokines in AR. A, The protein expression levels of IL-36 cytokines were elevated in AR and AR with asthma compared with controls. B, The ratio of IL-36γ/IL-36Ra protein level and the ratio of IL-36γ/IL-38 protein level in different groups. $^{*}P < 0.05$, compared with the control group.

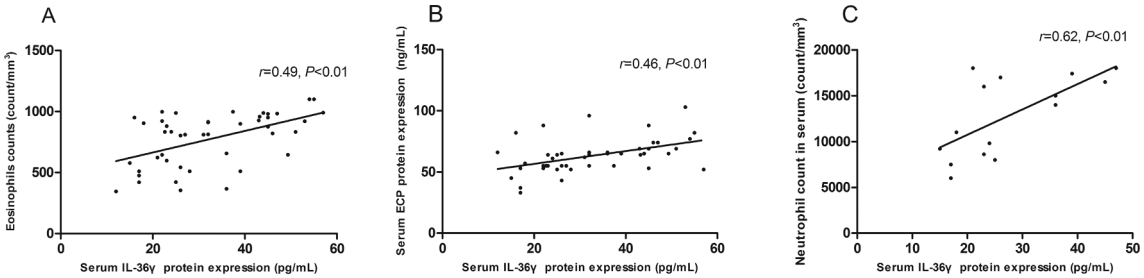

Fig. 3. Correlation between serum IL-36γ levels and eosinophils counts and ECP concentration in AR group were shown in A and B. Correlation between serum IL-36γ levels neutrophil count ($r = 0.58$, $P < 0.01$) in AR with asthma group were shown in C.

analysis. A P value of less than .05 was defined as statistically significant.

3. Results

3.1. The mRNA and protein expression of IL-36 family cytokines and IL-36R in AR

The demographic characteristics of study subjects are listed in Table 1. According to the occurrence of asthma, the patients were divided into AR group, AR with asthma group, and control group. Our data showed that the eosinophil number, total IgE, and eosinophil cationic protein (ECP) levels were significantly higher in the AR group compared with the control group, especially in AR with asthma group. We also found that the neutrophil count in AR with asthma group was significantly higher compared with AR group and control group, whereas the neutrophil count between AR and control group had no difference.

Our results found that both mRNA and protein expression of serum IL-36 family cytokines (IL-36α, IL-36β, IL-36γ, IL-36Ra, and IL-38) and IL-36R were significantly increased in AR compared with normal controls, especially in AR with asthma group. Of those cytokines, IL-36γ expression was the highest. We also found that higher relative mRNA and protein expression of IL-36γ/IL-36Ra and IL-36γ/IL-38 in AR compared with normal controls, especially in AR with asthma group (Figs. 1 and 2). Our results showed that serum IL-36γ levels were significantly correlated with eosinophils counts ($r = 0.68$, $P < 0.01$) and ECP concentration ($r = 0.66$, $P < 0.01$) in AR group and neutrophil

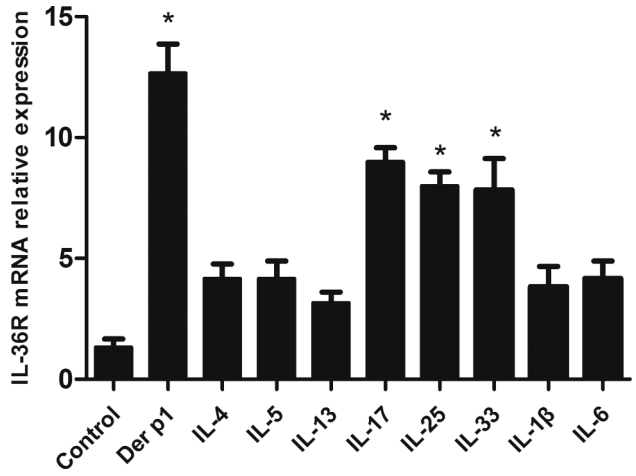

Fig. 4. The regulation of IL-36R mRNA expression from peripheral blood eosinophils (1.5 × 105/0.2 mL/well) after 15 h stimulation by various stimulators from AR donors. The experiments were repeated for three times. $^{*}P < 0.05$, compared with the control group. $^{*}P < 0.05$, compared with the control group.

count ($r = 0.58$, $P < 0.01$) in AR with asthma group (Fig. 3).

3.2. IL-36R expression and regulation by eosinophils in AR

Our results found that the mRNA of IL-36R by eosinophils in AR were significantly increased compared with normal controls (Fig. 1). We also stimulated eosinophils using different stimulators and the results showed that Der p1, IL-17, IL-25, and IL-33 promote the IL-36R mRNA expression by eosinophils (Fig. 4). We also found that AR with asthma showed the similar results with AR patients (Data not shown).

3.3. Role of IL-36γ in the function eosinophils

Our results showed that IL-36γ delayed spontaneous eosinophil death compared with controls (cell viability, 72.3% *vs* 35.4%) and the effect was inhibited when U0126 and SB203580 were added, suggesting that p38MAPK and MEK pathway were involve in the regulation of eosinophil death (Fig. 5A). We also found that IL-36γ promote expression of cluster of differentiation 18 (CD-18) and intercellular adhesion molecule 1 (ICAM-1) and inhibit ICAM-3 and L-selectin expression, proving that IL-36γ plays positive role in eosinophil adhesion (Fig. 5B). Our data also showed that IL-36γ induced the migration and activation of eosinophils (Fig. 5C). These effects were significantly inhibited by U0126 and SB203580 were added, suggesting that p38MAPK and MEK pathway were also engaged in the adhesion, migration and activation of eosinophils. We also found that IgG alone or all inhibitors alone (BAY, U, SP, SB, LY, AG) produced similar effect as control (Data not shown).

3.4. Regulation of IL-36γ expression in HNECs

We stimulated HNECS using different stimulators and the results showed that IL-17, IL-25, and IL-33 promoted the IL-36γ mRNA expression by HNECs (Fig. 6).

4. Discussion

In the present study, we first provided evidence that the expression of IL-36 family members were up-regulated significantly in AR, especially in patients with asthma. In the IL-36 family members, IL-36γ expression was the most abundant. We also found positive correlation between IL-36γ levels and eosinophils counts as well as ECP concentration. These findings suggested that IL-36γ may play important roles in the function of eosinophils. Besides, we also found that IL-36γ expression was correlated the neutrophil count in AR with asthma group, which is in line with other studies [22].

Next, we studied the expression of IL-36R using purified eosinophils. Our result first showed that IL-36R were expressed by eosinophils and up-regulated after stimulation by Der p1, IL-17, IL-25, IL-33. Previous studies had no report on the IL-36R expression by eosinophils. However, Liu's study showed that IL-36R expression on peripheral neutrophils could be up-regulated by IL-1b, IL-6, and Der p1, suggesting that IL-36R expression by eosinophils and neutrophils may have similar pathways [17].

To explore the direct effect of IL-36γ on the eosinophils, we performed series experiments. We found that IL-36γ promoted the survival of eosinophil and p38MAPK and MEK pathway mediated this effect. In the adhesion assay, we explore the effect of IL-36γ on common adhesion molecules. Previous studies had confirmed that ICAM-1 and integrin were deeply involved in the recruitment and trans-endothelial migration of eosinophils whereas ICAM-3 is highly expressed on resting eosinophil surface [23–25]. Consistently, we found that IL-36γ increased the expression of ICAM-1 and CD18 and inhibited ICAM-3 and L-selectin expression. In eosinophil migration assay, we first provide evidence that IL-36γ had direct effect on migration and activation of eosinophils. We also found that IL-36γ mediated adhesion and activation of eosinophils through p38MAPK and MEK pathway, which was not fully consistent with previous studies [26,27].

Finally, we investigated the expression of IL-36γ in human nasal epithelial cells. Our results suggested that IL-36γ mRNA expression in HNECs was up-regulated by IL-17, IL-25, IL-33, which were similar with the reports in bronchial epithelial cells [6].

So far, few studies explored the relationship between IL-36 and eosinophils. In Agnieszka's study, they provided preliminary evidence that IL 36 may play a relevant role of enrolling eosinophils and neutrophils in DH, BP, and PV and finally provoke tissue injury [28]. Another study had proved that the expression of IL-36γ by the lung is up-regulated in asthma susceptible mice compared to asthma resistant C3H/HeJ mice in allergen induced mice model, suggesting that IL-36γ play a role in allergic inflammation [29]. In their study, recombinant IL-36γ given intratracheally results in neutrophil influx, but not eosinophilic influx in the lungs of mice. Inconsistently, our study first proved that IL-36γ promotes the survival, adhesion, and activation of eosinophils. This discrepancy between studies may be due to different disease backgrounds and the difference which may be caused by *in vitro* and *in vivo* studies. Therefore, our results should be further proved by animal model.

Our study also had some limitations. First, we only focused the expression and regulation of IL-36γ on eosinophils and HNEC, however, IL-36γ may also be produced by other cells. Second, animal model were not established in this study. Third, nasal turbinate mucosa were not used in this study.

In summary, our study shows that up-regulation of IL-36γ exaggerates eosinophilic inflammation in AR by promoting the survival, adhesion, migration and activation of eosinophils. Therefore, targeting IL-36γ represents a promising novel pathway to control inflammation in AR.

Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

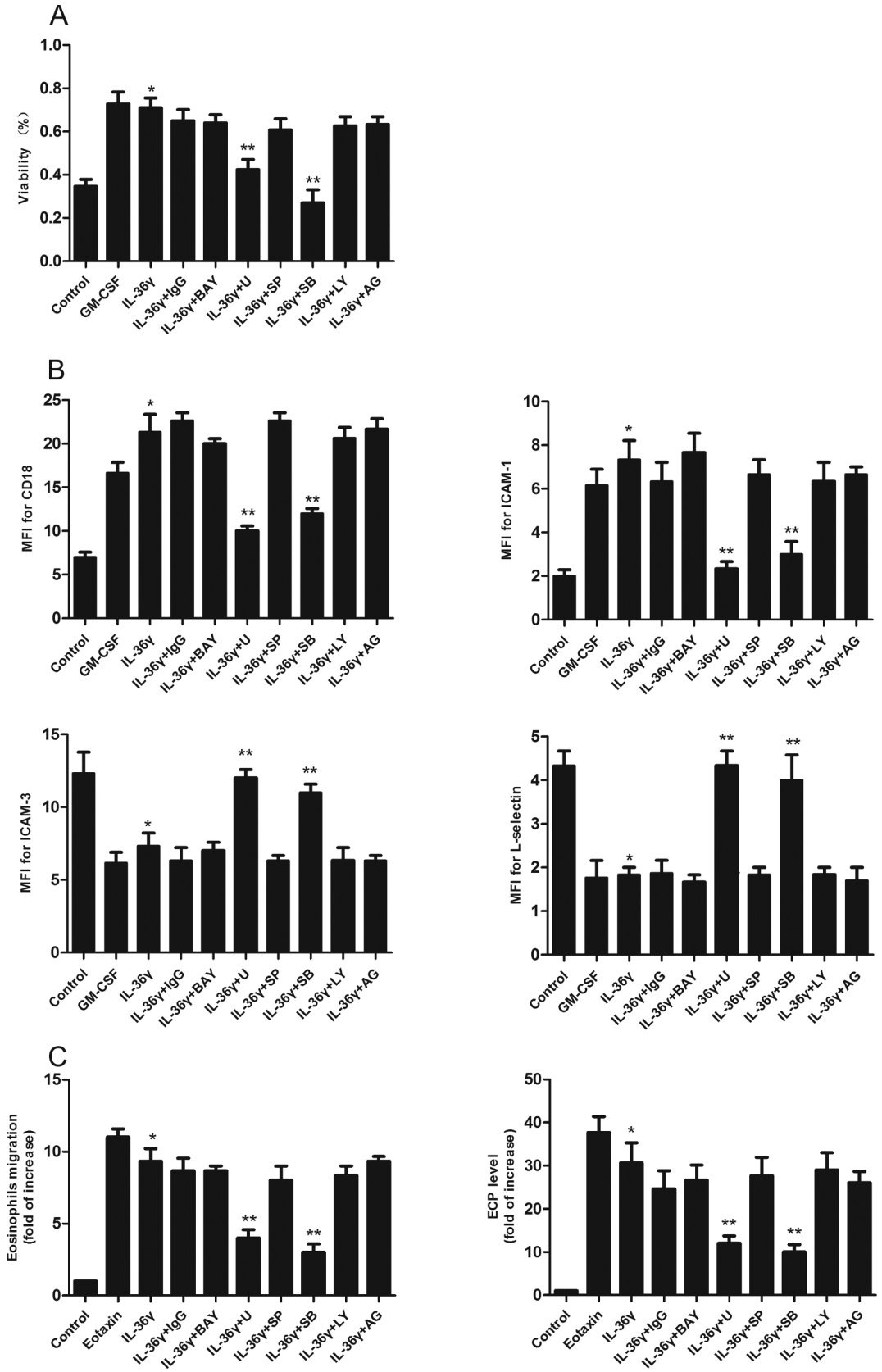

Fig. 5. The viability of eosinophils (A) after 24 h stimulation by IL-36γ and/or signaling pathway inhibitors. The expression of adhesion molecules such as CD18, ICAM-1(F), ICAM-3 and L-selectin were shown as MFI (B) after 15 h stimulation by IL-36γ and/or signaling pathway inhibitors. IL-36γsignificantly enhanced EOS migration and activation (C) after 6 h stimulation. $^{*}P < 0.05$, compared with the control group. $^{**}P < 0.05$, compared with the IL-36γ group. BAY, 1 μM, IκB-α phosphorylation inhibitor; U, 1 μM, MEK inhibitor, SP, 3 μM, Jun N-terminal kinase (JNK) inhibitor, SB, 7.5 μM, p38MAPK inhibitor, LY, 5 μM, phosphatidylinositol 3-OH kinase (PI3K)/Akt inhibitor, AG, 10 μM, Janus kinase (JAK) inhibitor.

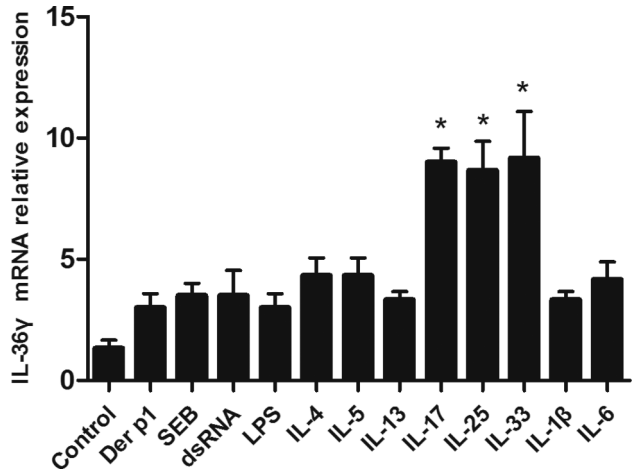

Fig. 6. The regulation of IL-36γ mRNA expression on Human nasal epithelial cells by various stimulators. $^{*}P < 0.05$, compared with the control group. LPS, lipopolysaccharides, SEB, staphylococcal enterotoxin B.

References

[1] H.F. Rosenberg, S. Phipps, P.S. Foster, Eosinophil trafficking in allergy and asthma, J. Allergy Clin. Immunol. 119 (2007) 1303–1310.

[2] C. Garlanda, C.A. Dinarello, A. Mantovani, The interleukin-1 family: back to the future, Immunity 39 (2013) 1003–1018.

[3] D. Boraschi, A. Tagliabue, The interleukin-1 receptor family, Semin. Immunol. 25 (2013) 394–407.

[4] C. Gabay, J.E. Towne, Regulation and function of interleukin-36 cytokines in homeostasis and pathological conditions, J. Leukoc. Biol. 97 (2015) 645–652.

[5] M.S. Gresnigt, F.L. van de Veerdonk, Biology of IL-36 cytokines and their role in disease, Semin. Immunol. 25 (2013) 458–465.

[6] R.T. Chustz, D.R. Nagarkar, J.A. Poposki, et al., Regulation and function of the IL-1 family cytokine IL-1F9 in human bronchial epithelial cells, Am. J. Respir. Cell. Mol. Biol. 45 (2011) 145–153.

[7] Y. Carrier, H.L. Ma, H.E. Ramon, L. Napierata, et al., Interregulation of Th17 cytokines and the IL-36 cytokines in vitro and in vivo: implications in psoriasis pathogenesis, J. Invest. Dermatol. 131 (2011) 2428–2437.

[8] S. Frey, A. Derer A, M.E. Messbacher, et al., The novel cytokine interleukin-36alpha is expressed in psoriatic and rheumatoid arthritis synovium, Ann. Rheum. Dis. 73 (2013) 1569–1574.

[9] P. Wang, B. Meinhardt, R. Andre, et al., The interleukin-1-related cytokine IL-1F8 is expressed in glial cells, but fails to induce IL-1beta signalling responses, Cytokine 29 (2005) 245–250.

[10] D. Magne, G. Palmer, J.L. Barton, et al., The new IL-1 family member IL-1F8 stimulates production of inflammatory mediators by synovial fibroblasts and articular chondrocytes, Arthritis. Res. Ther. 8 (2006) R80.

[11] A.M. Foster, J. Baliwag, C.S. Chen, et al., IL-36 promotes myeloid cell infiltration, activation, and inflammatory activity in skin, J. Immunol. 192 (2014) 6053–6061.

[12] J.E. Towne, K.E. Garka, B.R. Renshaw, et al., Interleukin (IL)-1F6, IL-1F8, and IL-1F9 signal through IL-1Rrp2 and IL-1RAcP to activate the pathway leading to NF-kappaB and MAPKs, J. Biol. Chem. 279 (2004) 13677–13688.

[13] Y. Shaik, G. Sabatino, G. Maccauro, et al., IL-36 receptor antagonist with special emphasis on IL-38, Int, J, Immunopathol. Pharmacol. 26 (2013) 27–36.

[14] F.L. van de Veerdonk, A.K. Stoeckman, G. Wu, et al., IL-38 binds to the IL-36 receptor and has biological effects on immune cells similar to IL-36 receptor antagonist, Proc. Natl. Acad. Sci. USA 109 (2012) 3001–3005.

[15] R. Debets, J.C. Timans, B. Homey, et al., Two novel IL-1 family members, IL-1 delta and IL-1 epsilon, function as an antagonist and agonist of NF-kappa B activation through the orphan IL-1 receptor-related protein, J. Immunol. 167 (2001) 1440–1446.

[16] A. Johnston, X. Xing, L. Wolterink, et al., IL-1 and IL-36 are dominant cytokines in generalized pustular psoriasis, J. Allergy Clin. Immunol. 140 (2016) 109–120.

[17] H. Wang, Z.Y. Li, W.X. Jiang, et al., The activation and function of IL-36γ in neutrophilic inflammation in chronic rhinosinusitis, J. Allergy. Clin. Immunol. 141 (2018) 1646–1658.

[18] J.L. Brozek, J. Bousquet, C.E. Baena-Cagnani, et al., Allergic Rhinitis and its impact on Asthma (ARIA) guidelines: 2010 revision, J. Allergy Clin. Immunol. 126 (2010) 466–476.

[19] X.H. Zhang, Y.N. Zhang, H.B. Li, et al., Overexpression of miR-125b, a novel regulator of innate immunity, in eosinophilic chronic rhinosinusitis with nasal polyps, Am. J. Respir. Crit. Care. Med. 185 (2012) 140–151.

[20] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method, Methods 25 (2001) 402–408.

[21] M.B. Grotta, D.M. Squebola-Cola, A.A. Toro, et al., Obesity increases eosinophil activity in asthmatic children and adolescents, BMC Pulm. Med. 18 (2013) 39.

[22] R.A. Ramadas, S.L. Ewart, Y. Iwakura, A.M. Levine, et al., IL-36alpha exerts pro-inflammatory effects in the lungs of mice, PLoS ONE 7 (2012) e45784.

[23] M.W. Johansson, Activation states of blood eosinophils in asthma, Clin. Exp. Allergy 44 (2014) 482–498.

[24] S.H. Ko, J.I. Jeon, Y.J. Kim, et al., Helicobacter pylori outer membrane vesicle proteins induce human eosinophil degranulation via a β2 Integrin CD11/CD18- and ICAM-1-dependent mechanism, Mediators. Inflamm. 2015 (2015) 301716.

[25] J.M. Kessel, J.E. Gern, R.F. Vrtis, et al., Ligation of intercellular adhesion molecule 3 inhibits GM-CSF production by human eosinophils, J. Allergy Clin. Immunol. 111 (2003) 1024–1031.

[26] C.K. Wong, W.K. Ip, C.W.K. Lam, IL-3, 5 and GM-CSF-induced adhesion molecule expression on eosinophils by p38 MAPK and NF-jB, Am. J. Respir. Cell Mol. Biol. 29 (2003) 133–147.

[27] G.T. Kampen, S. Stafford, T. Adachi, et al., Eotaxin induces degranulation and chemotaxis of eosinophils through the activation of ERK2 and p38 mitogen-activated protein kinases, Blood 95 (2000) 1911–1917.

[28] A. Żebrowska, A. Woźniacka, K. Juczyńska K, et al., Correlation between IL36α and IL17 and activity of the disease in selected autoimmune blistering diseases, Mediators Inflamm. 2017 (2017) 8980534.

[29] Ra. Ramadas, X. Li, D.M. Shubitowski, et al., IL-1 receptor antagonist as a positional candidate gene in a murine model of allergic asthma, Immunogenetics 58 (2006) 851–855.