

Association analysis of high-mobility group box-1 protein 1 (HMGB1)/toll-like receptor (TLR) 4 with nasal interleukins in allergic rhinitis patients

Xuewei Zhu^a, Jianan Cong^b, Ben Yang^{c,*}, Yuxin Sun^{a,*}

^a Department of Otolaryngology Head & Neck Surgery, China-Japan Union Hospital of Jilin University, Changchun 130033, Jilin, PR China

^b Department of Ophthalmology, Changchun City Central Hospital, Changchun 130051, Jilin, PR China

^c Department of Ophthalmology, China-Japan Union Hospital of Jilin University, Changchun 130033, Jilin, PR China

ARTICLE INFO

Keywords:

High-mobility group box-1 protein 1 (HMGB1)
Toll-like receptor (TLR)-4
Interleukins (ILs)
Allergic rhinitis (AR)

ABSTRACT

Background: High mobility group box 1 (HMGB1) acts as an inflammatory mediator initiator, and has shown to increase in nasal secretions of allergic rhinitis (AR) patients. The purpose of the present study was to investigate the association of HMGB1 and its receptor, toll-like receptor (TLR) 4 with proinflammatory interleukins in AR patients.

Methods: The interleukin (IL) -4, -5, -13, -17A, -10 in nasal lavage fluid and the serum IgE were examined with enzyme-linked immunosorbent assay (ELISA) in 125 AR patients or 87 healthy subjects (as control). The mRNA levels of HMGB1, receptor for advanced glycation end products (RAGE) and TLR 2, 3 and 4 were examined in these participants with real-time quantitative PCR (RT-qPCR) method. Spearman rank correlation was adopted to analyze the relationship between any two factors of clinical characteristics and laboratory-examined biomarkers.

Results: IL-4, -5, -13 and -17A were significantly higher, whereas IL-10 was markedly lower in the nasal lavage fluid samples from AR patients, compared with the control subjects. High expression of HMGB1, TLR2 and TLR4 were observed in the nasal brushing samples from these AR patients, whereas there was no significant difference for the mRNA levels of TLR3 and RAGE. Besides, HMGB1/TLR4 mRNA levels correlated positively with IL-4, -5, -13 and -17A, whereas negatively with IL-10. A linear regression of HMGB1/TLR4 was also found positively with IL-4, -5 (only for TLR4), -13 or -17A, whereas negatively with IL-10.

Conclusion: High mRNA levels of HMGB1 and TLR4 were found in the nasal brushing samples in AR patients, correlating positively with IL-4, -5, -13 or -17A, whereas negatively with IL-10. HMGB1/TLR4 signaling pathway might be well responsive targets as immunotherapy for allergic rhinitis.

1. Introduction

Allergic rhinitis (AR) is the most prevalent atopic disease in the world, affecting 10–40% of the population [1,2]. It is characterized by an IgE-mediated inflammation of the mucous membranes lining the nose, in response to an allergen exposure [3], and is clinically defined as a chronic upper respiratory disease, with nasal obstruction, sneezing, rhinorrhea, and suspected allergen-associated pruritus. There is a high comorbidity of AR with asthma [4] and a mutual promotion to the disease progression for each other [5]. Post-exposure to allergens, the nasal mucosa initiates series of pathophysiological processes, such as the activation and migration of effector cells, the release of mediators, chemokines and cytokines from inflammatory cells, and the damage to the nasal epithelium and nerve endings [6]. Highly-sensitive type 2, 1,

and 17 T-helper (Th) cells play central roles in the pathogenesis of allergic airway inflammation [7,8]. Th2 cells-secreted interleukin (IL) 4, IL-5, and IL-13 amplify allergic inflammation; Th1 cells-secreted interferon (IFN) promote neutrophil infiltration [9]; Th17 cells-produced IL-17A, Th17F, and IL-21 enhance the development of chronic airway inflammation. On the other side, reduced activity of regulatory T cells (Treg) and regulatory B cells (Breg) were observed in AR [10,11]. Therefore, proinflammatory factors mediate the development and the persistence of nasal inflammation, including AR [12]. However, the orchestrating signaling pathways underlining these processes were not fully understood.

High mobility group box 1 (HMGB1) is a nucleus-abundantly-expressed non-histone chromosomal binding protein, acts as an initiator of inflammatory factors [13]. HMGB1 can be modified into three

* Corresponding authors at: Department of Otolaryngology Head & Neck Surgery, China-Japan Union Hospital of Jilin University, 126 Xian Street, Changchun 130033, Jilin, PR China (Y. Sun).

E-mail addresses: yangben@jlu.edu.cn (B. Yang), syxmy2015@sina.com (Y. Sun).

<https://doi.org/10.1016/j.cyto.2019.154880>

Received 28 June 2019; Received in revised form 23 September 2019; Accepted 7 October 2019

1043-4666/ © 2019 Published by Elsevier Ltd.

Table 1
Clinical characteristics and the average levels of laboratory factors.

Characteristics	Ctrl group (N = 87)	AR group (N = 125)
Age (years)	28.32 ± 5.3	29.63 ± 5.09
Gender (%Male)	54.02	54.40
Weight (kg)	58.87 ± 11.37	59.63 ± 12.26
Height (cm)	166.07 ± 10.94	165.52 ± 10.16
IgE (KU/L)	1.26 ± 0.17	2.52 ± 0.54
IL-4 (pg/ml)	2.56 ± 0.22	4.41 ± 0.65
IL-5 (pg/ml)	1.67 ± 0.14	2.88 ± 0.45
IL-13 (pg/ml)	12.69 ± 1.28	18.7 ± 2.52
IL-17A (pg/ml)	9.69 ± 0.95	14.41 ± 2.12
IL-10 (pg/ml)	1.31 ± 0.12	1.06 ± 0.13
Nasal HMGB1 (AR/Ctrl)	1.00 ± 0.15	1.43 ± 0.32
Nasal RAGE (AR/Ctrl)	1.00 ± 0.18	1.05 ± 0.27
Nasal TLR2 (AR/Ctrl)	1.06 ± 0.38	1.23 ± 0.23
Nasal TLR3 (AR/Ctrl)	1.03 ± 0.19	1.02 ± 0.22
Nasal TLR4 (AR/Ctrl)	1.02 ± 0.32	1.23 ± 0.24

isoforms, termed all-thiol HMGB1, disulfide HMGB1 (partially oxidized), and oxidized HMGB1 according to the status of its three redox-sensitive cysteine residues (C23, C45, and C106) [14,15]. HMGB1 has been reported to interact with its receptors, including the receptor for advanced glycation end products (RAGE) and toll-like receptor (TLR) 2/4, and to induce extracellular signal-regulated signaling, which triggers cytokine production [16]. Studies have shown that the HMGB1 level in nasal secretions of AR patients is significantly increased [17]. Glycyrrhizic acid (GLT) reduces the intensity of nasal allergies by binding to HMGB1; budesonide nasal spray can also reduce the level of HMGB1 in the nasal secretions of AR patients. The GLT- or budesonide-mediated HMGB1 inhibition reduced the eosinophil activity and the release of

inflammatory mediators in AR nasal mucosa and ameliorated nasal symptoms [18,19]. In particular, dust mite allergen stimulated the nasal mucosa of mice to produce a large amount of HMGB1, which induced nasal allergies. The HMGB1- receptor for RAGE gene eliminates the intensity of allergic rhinitis caused by dust mite stimulation in mice. The severity of allergic rhinitis caused by dust mite stimulation is significantly down-regulated in mice [18]. Therefore, we deduced that HMGB1 might be an inflammation initiator to the nasal inflammation in AR.

In the present study, we examined the interleukin (IL) -4, -5, -13, -17A, -10 in nasal lavage fluid and the mRNA levels of HMGB1, RAGE, TLR 2, 3 and 4 in the nasal brushing samples from AR and control participants. Spearman rank correlation and linear regression were adopted to analyze the relationship between any two factors of clinical characteristics and laboratory-examined biomarkers. Our study implies that HMGB1/TLR4 signaling pathway might be well responsive targets as immunotherapy for allergic rhinitis.

2. Methods

2.1. Allergic rhinitis patients, nasal sample collection

One hundred and twenty-five allergic rhinitis patients and 87 healthy control subjects were enrolled for the present study, from 2016 May to 2018 August. Smoking habit, other acute or chronic infection or inflammatory diseases in nasal or upper respiratory tract were excluded for each participant. The diagnosis of each AR patient was made under the guidance of the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines-2016/2010 revision [20,21]. Our study was approved by the institutional ethics committees at China-Japan Union Hospital of Jilin

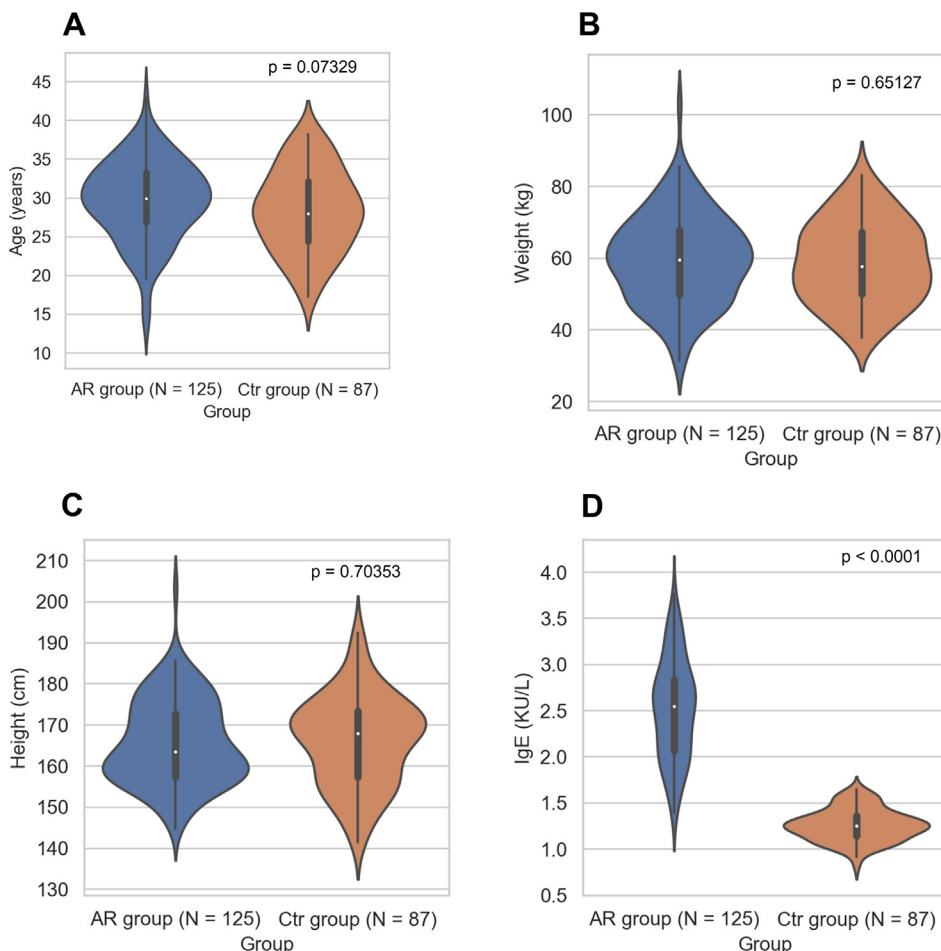


Fig. 1. Compare clinical characteristics between allergic rhinitis patients and healthy control subjects. The age (A), weight (B), height (C) and serum IgE level (KU/L) (D) for allergic rhinitis patients (N = 125) and normal control subjects (N = 87) were plotted with violin plots. A box plot inside violin plots was set to indicate the maximum value, 75%, 50% and 25%-quantile values and the minimum value were indicated as the top whisker, the top border, the middle line, the bottom border, and the bottom whisker, respectively. The probability density of different values was indicated by the curve, outside the box plot, of a smoothed kernel density estimator. Statistical p values were annotated respectively in each figure, and a statistical significance was considered when a p-value less than 0.05.

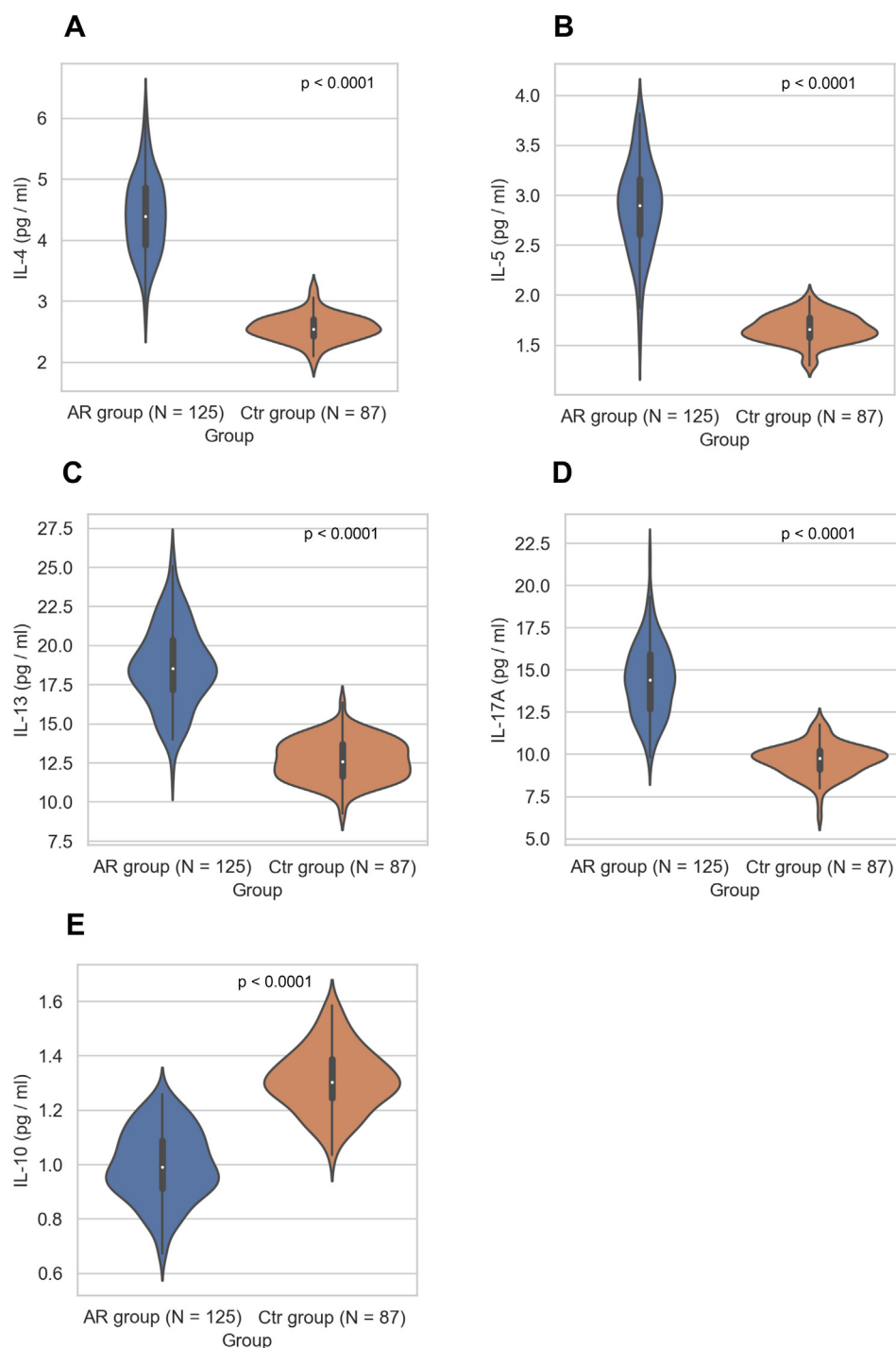


Fig. 2. Different interleukin levels in the nasal lavage fluid from allergic rhinitis patients and healthy control subjects. The nasal lavage fluid was collected from allergic rhinitis patients or healthy subjects and then was examined for the level of interleukin (IL)-4 (A), IL-5 (B), IL-13 (C), IL-17A (D) or IL-10 (E). Data were plotted with violin plots, in which, the maximum value, 75%, 50% and 25% quantile values and the minimum value were indicated as the top whisker, the top border, the middle line, the bottom border, and the bottom whisker, respectively of the box plot inside. The probability density of different values was indicated by the curve, outside the box plot. Statistical p values were annotated, respectively in each figure.

University (Chang Chun, Jilin 130033, PR China). Every participant was fully aware of this study and written informed consent was obtained from each of them. The age, gender, height, and weight of each participant was recorded in Table 1.

Serum IgE was collected from the peripheral venous blood (5 mL) without any anticoagulants, post standing at 4 °C for 4 h and then post the centrifuging with 1500g for 30 min. The nasal lavage was collected with an atomized nasal washing machine (Neb-nid, Flaem Nuova SPA, Italy), by filling and washing nasal cavity with 6 mL natural saline (0.9%) [22]. The collected lavage samples were centrifuged at 4 °C with 3000g for 15 min to remove any cell debris and then were filtered with a micro-filter (0.6 µm, Millipore, Bedford, MA, USA) to remove mucus. The supernatant was then collected and was stored at 4 °C within 12 h

for the IL examination or was stored at −80 °C for further usage.

Post the nasal lavage collection; nasal brushing was performed with a channel cleaning brush, which is designed for fiberoptic bronchoscopes (model BW-15SH, Olympus, Japan). Nasal cells were collected under direct visualization, using a headlamp, by a few backward-forward and rotatory movements. The brushing sample adhering to the brush was then resuspended in 2 mL ice-cold natural saline and was immediately for the mRNA isolation.

2.2. Enzyme-linked immunosorbent assay (ELISA) for interleukins

ELISA was performed with the commercial reagent kit (Qayee-Bio, Shanghai, China) for IgE, IL-4, −5, −10, −13 or −17A under the

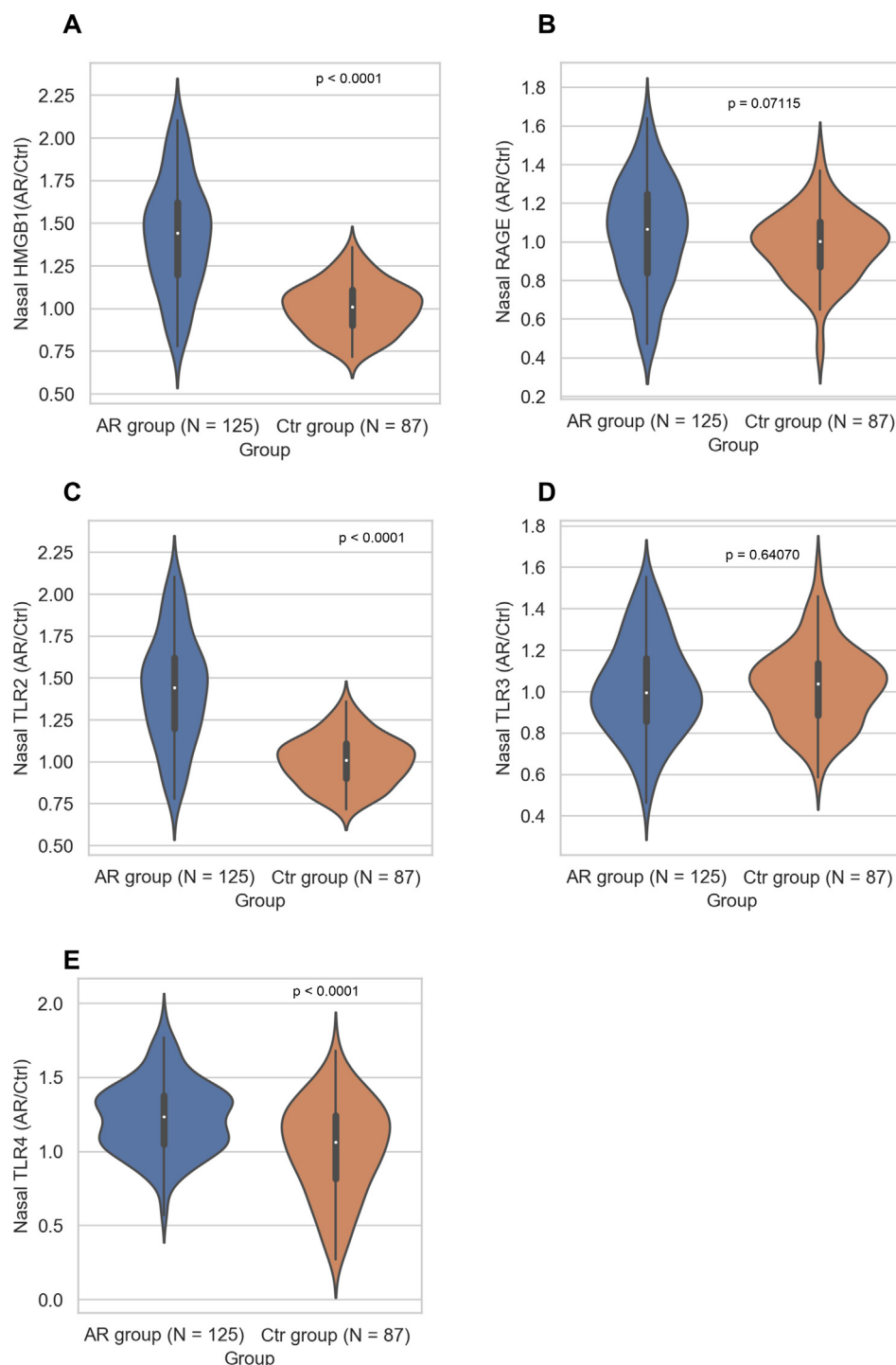


Fig. 3. mRNA levels of HMGB1 and the receptors of HMGB1 in the nasal brushing samples from allergic rhinitis patients and normal control subjects. The mRNA levels of the receptor for advanced glycation end-products (RAGE) (A), HMGB1 (B), toll-like receptor (TLR)2 (C), TLR3 (D), or TLR4 (E) in the nasal brushing samples were quantified with real-time quantitative PCR. The mRNA level for each target was corrected with the relative level to β -actin and then was presented as the relative level in AR group to control group. Data were plotted with violin plot. Statistical p values were annotated, respectively.

guidance of the manufacturer's instructions. The examination for each molecule was independently repeated for three times and was presented as an average level.

2.3. mRNA isolation and real-time quantitative PCR (RT-qPCR)

Rapid mRNA purified from total RNA: Dynabeads® mRNA Purification Kit (Thermo Scientific, Rockford, IL, USA) was utilized to isolate mRNA samples from the nasal brushing samples under the

guidance of the kit's manual. The mRNA sample from each nasal brushing sample was eluted from the filter with 60 μ l sterile ultra-pure water containing RNasin® Plus RNase Inhibitor (Promega, Madison, WI, USA), and then was stored at -80°C before use. Relative mRNA levels of HMGB1, RAGE, TLR2, TLR3, and TLR4 were performed with the specially paired primers for each target molecule and with the QuantiTect SYBR Green PCR kit (QIAGEN GmbH, Hilden, Germany) on LightCycler 2.0 (Roche Diagnostics, Mannheim, Germany) under the condition suggested by the kit. The sequences of primers used here were

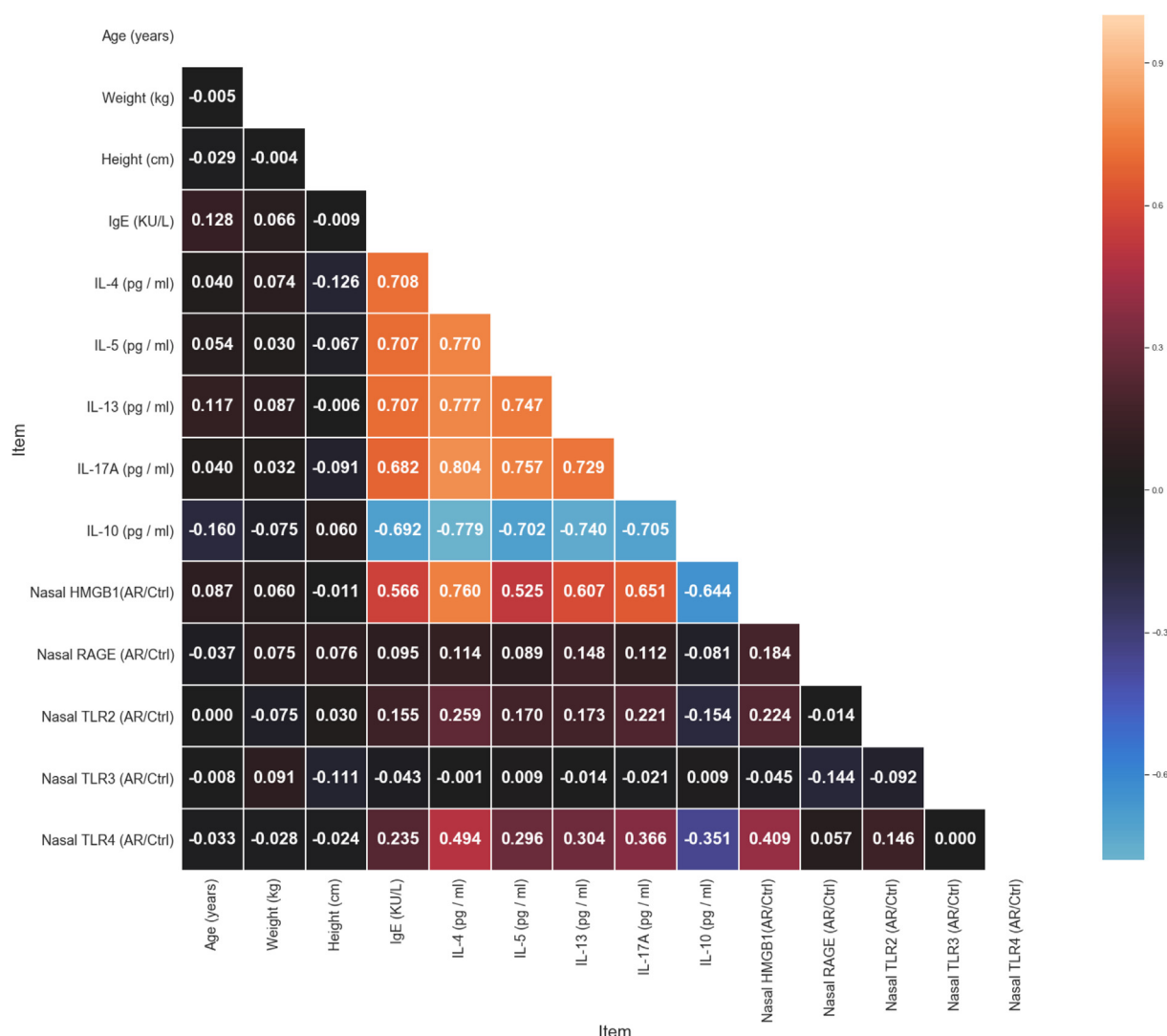


Fig. 4. Correlation between the levels of ILs and the mRNA levels of HMGB1/TLR-4 from allergic rhinitis patients and healthy control subjects. Spearman correlation was performed to analyze the correlation between every two items of the clinical characteristics, the nasal interleukin levels and the mRNA levels in the nasal brushing samples from allergic rhinitis patients. R^2 for every two factors was presented in each data cell, with the x-axis and y-axis labels, $R^2 \geq 0.25$ was set as a threshold for a positive correlation.

available upon a request. β -actin was taken as an internal mRNA control for each target molecule. The relative mRNA level of each molecule was presented as a relative value to the β -actin value, and then relative to the value in the control group.

2.4. Statistical analysis

Statistical analysis was performed with SPSS (version 18.0, IBM SPSS Inc. Armonk NY, USA) and with GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, California, USA). The unpaired t -test to analyze the difference of each clinical characteristic or laboratory biomarker between the two groups. The correlation and the regression between any two items were performed with the spearman rank correlation or linear regression. A statistical significance was considered when a p -value less than 0.05.

3. Results

3.1. Clinical characteristics and the nasal levels of inflammatory interleukins for allergic rhinitis (AR) patients

One hundred and twenty-five allergic rhinitis patients and 87

healthy control subjects that were enrolled from 2016 May to 2018 August included in this study. The percentage of males, the average level/standard deviation of age (years), weight (kg) and height (cm) for the participants in both groups were listed in Table 1. All values in both groups were plotted with violin plot for age (Fig. 1A), weight (Fig. 1B), height (Fig. 1C) and serum IgE level (Fig. 1D), respectively. As indicated, there was no significant difference in the age, weight and height between the two groups. A markedly high IgE level was observed for AR patients.

The AR-associated ILs in nasal lavage fluid samples were quantified with ELISA. It was indicated in Fig. 2 that all data were normally distributed for both groups. As shown in Table 1, there were significantly higher levels of IL-4 (4.41 ± 0.65 vs. 2.56 ± 0.22), IL-5 (2.88 ± 0.45 vs. 1.67 ± 0.14), IL-13 (18.7 ± 2.52 vs. 12.69 ± 1.28) and IL-17A (14.41 ± 2.12 vs. 9.69 ± 0.95), whereas a markedly lower level of IL-10 (1.06 ± 0.13 vs. 1.31 ± 0.12) in the AR group, compared with the control group. The statistical significance was annotated, respectively in Fig. 2A–E.

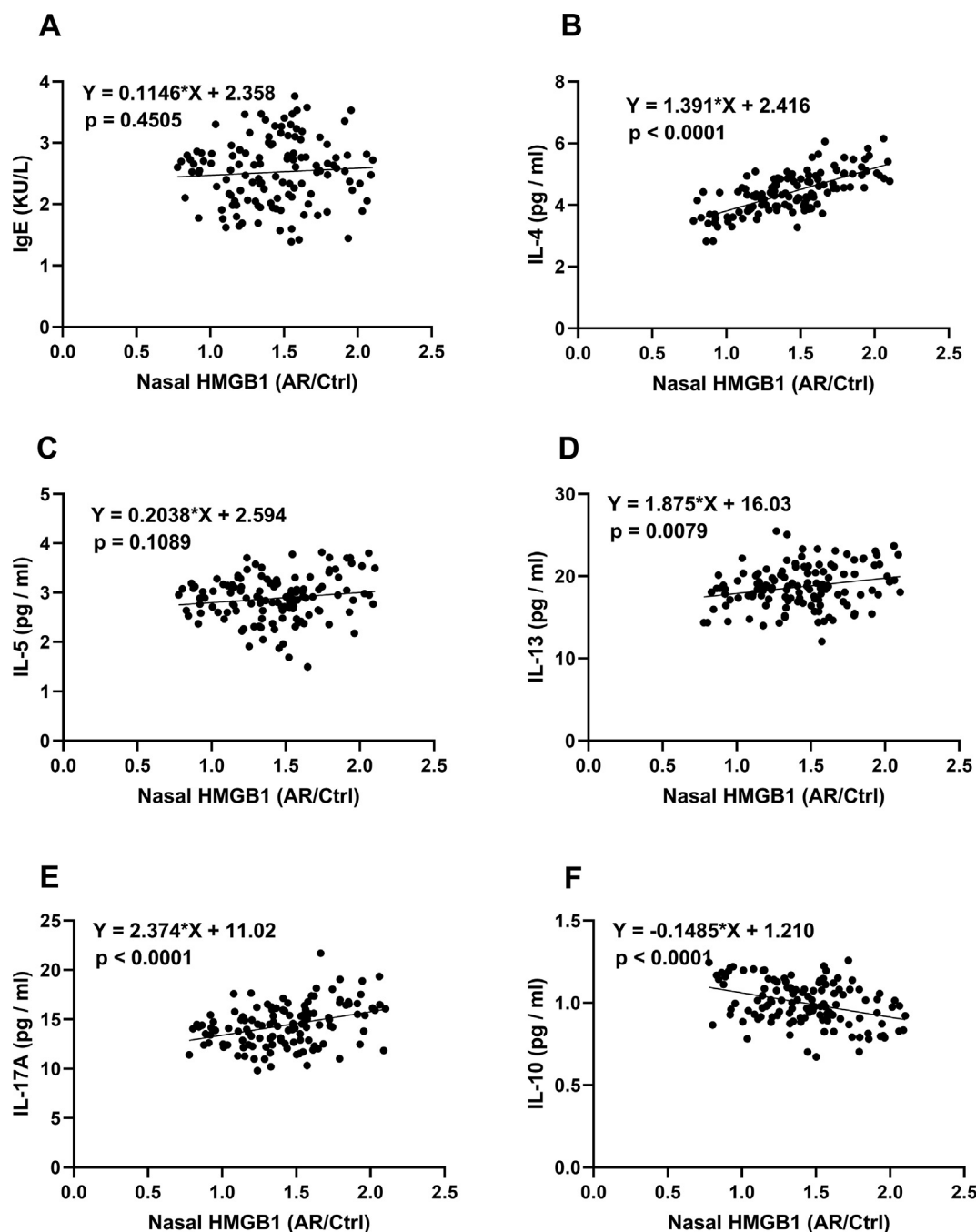


Fig. 5. Linear regression analysis of nasal brushing HMGB1 mRNA with nasal lavage ILs. The association of nasal brushing HMGB1 mRNA with nasal lavage ILs were analyzed via a linear regression model, in which, HMGB1 was taken as an independent variable and IgE or IL was taken as explanatory variables. The linear regression function and the p-value were annotated, respectively.

3.2. High expression of HMGB1 and its receptors in nasal brushing samples from AR patients

Increased nasal secretion of HMGB1 has been observed for AR patients [17]. Targeted inhibition to HMGB1 was also indicated to markedly ameliorate AR nasal symptoms [18,19]. To further investigate the possible role of HMGB1 in the nasal inflammation of AR, we examine the expression of HMGB1 and its major receptor of TLR2/4 and RAGE [16]. Here, we collected brushing samples from AR patients and quantified the nasal HMGB1 levels and then analyzed the association of nasal HMGB1 level with IL secretion for AR patients. The relative mRNA level of Nasal HMGB1 (AR/Ctrl) was 1.43 ± 0.32 in AR group, significantly higher than 1.00 ± 0.15 in control group

($p < 0.001$, Fig. 3A). Interestingly, the expression of HMGB1 receptors varied in the nasal brushing samples. The RAGE mRNA level was not different between AR and control groups (1.05 ± 0.27 vs. 1.00 ± 0.18 , $p = 0.07106$, Fig. 3B). However, significantly higher levels of TLR2 and TLR4, whereas not of TLR3, were observed in AR patients, compared with control subjects (Fig. 3C–E).

3.3. Association of nasal brushing HMGB1 and its receptors with nasal lavage ILs in AR patients

To investigate the role of HMGB1 for the nasal secretion of ILs for the AR patients, we then performed the spearman rank correlation for every two factors of the clinical characteristics and laboratory

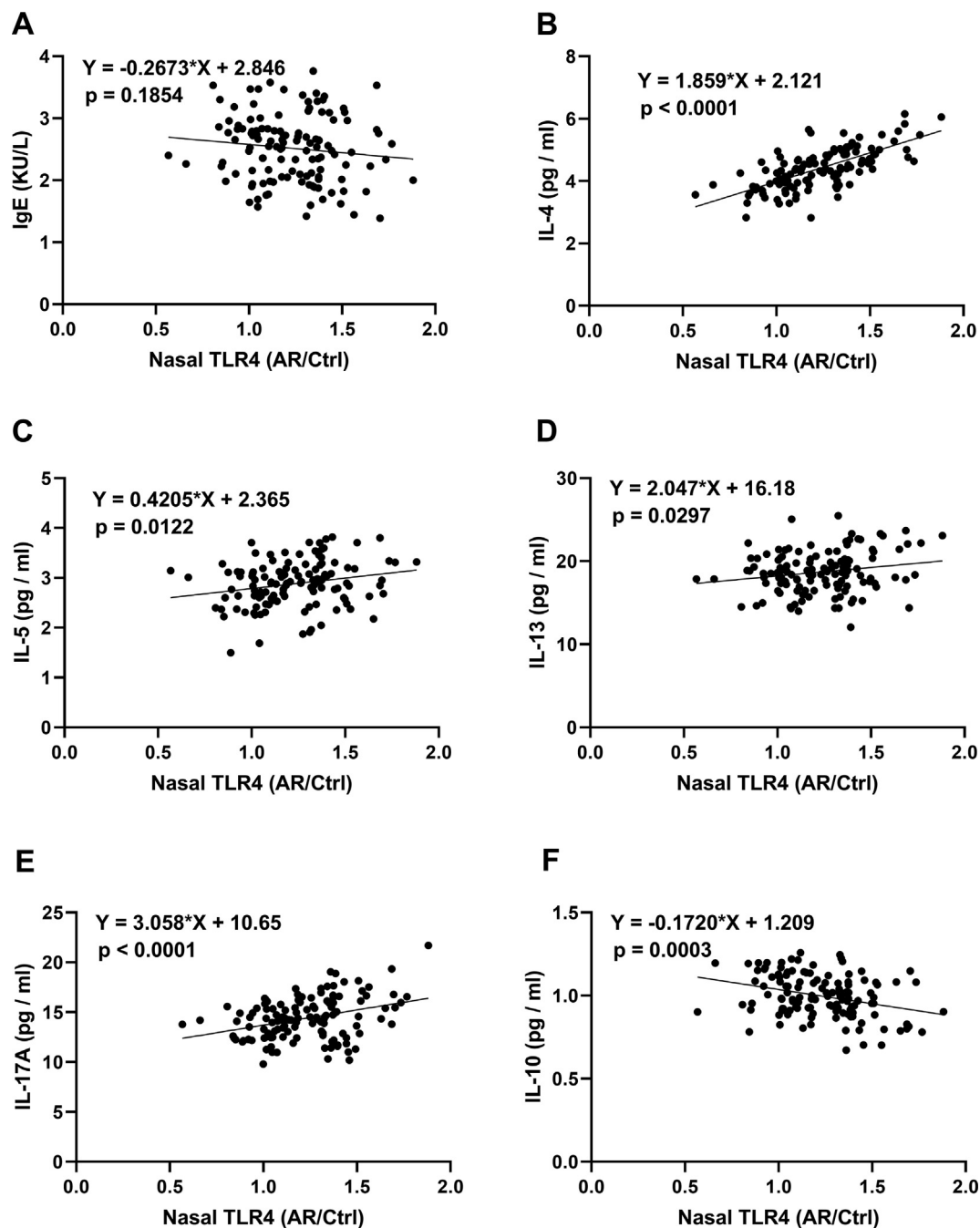


Fig. 6. Linear regression analysis of nasal brushing TLR4 mRNA with nasal lavage ILs. The association of nasal brushing TLR4 mRNA with nasal lavage ILs were analyzed via a linear regression model, in which, TLR4 was taken as an independent variable and IgE or IL was taken as explanatory variables. The linear regression function and the p-value were annotated, respectively.

molecular levels for AR patients. As shown in Fig. 4, Each value in the box with its x-axis label and y-axis label was the R^2 correlation value of Spearman rank correlation. The R^2 value of more than 0.25 was considered to present a significant association between the x-axis label and y-axis label. A high positive relationship was found between any two ILs of IL-4, IL-5, IL-13, and IL-17A and between IgE and any one of the ILs mentioned above, whereas IL-10 was found to negatively correlate with any of the above-mentioned ILs or IgE.

Moreover, a high positive correlation of HMGB1 was found with IL-4, IL-5, IL-13, IL-17A or IgE, the negative correlation was also observed between HMGB1 and IL-10. Interestingly, the association of HMGB1 receptors was not found as high as which of HMGB1 with any one of the ILs mentioned above. In particular, RAGE did not correlate with any of

ILs, with HMGB1, or with any one of the three TLRs. The correlation of TLR2 was significant with IL-4, whereas not significant with other ILs. However, an association of TLR4 was found positively with IL-4, IL-5, IL-13, IL-17A or IgE, or negatively with IL-10. It also positively correlated with HMGB1. Also, age, weight or height was not found to significantly correlate with anyone of above-mentioned laboratory factors. Of course, the negative control TLR, TLR3, neither associated with factors above.

Additionally, linear regression was performed to quantitatively analyze the dependence of AR-associated inflammatory factors on HMGB1 (Fig. 5A–F) or on TLR4 (Fig. 6A–F). There was no linear regression between HMGB1 and IgE level ($p = 0.4505$, Fig. 5A), or between HMGB1 and IL-5 ($p = 0.1089$, Fig. 5C). Fig. 5B indicated a

significant dependence of IL-4 on HMGB1 ($Y = 1.391 * X + 2.416$, $p < 0.001$). The dependence on HMGB1 was observed for the increase of IL-13 ($p = 0.0079$, Fig. 5D) and IL-17A ($p = 0.0079$, Fig. 5D), or for the decrease of IL-10 ($p < 0.001$, Fig. 5F). The dependence on TLR4 was also found for IL-4, IL-13, IL-17A and IL-10 (Fig. 6B, 6D, 6E and 6F), moreover, for IL-5 ($P = 0.0122$, Fig. 6C).

4. Discussion

High mobility group box 1 (HMGB1) acts as an initiator of inflammatory factors [13], via interacting with its receptors, including the receptor for advanced glycation end products (RAGE) and toll-like receptor (TLR) 2/4, to trigger cytokine production [16]. A significantly higher nasal secretion has been found in AR patients [17]. Nasal brushing has been adopted to collect the nasal inflammatory cells, in a variety of inflammatory diseases in the upper respiratory tract, including AR [20,23]. In the present study, we examined the mRNA level of HMGB1 and its receptors in the nasal brushing samples, and we found HMGB1 and its receptors, TLR 2 and TLR4, were also markedly higher at mRNA level in the nasal brushing samples from AR patients. Thus, both protein and mRNA levels of HMGB1 were confirmed to overexpress in nasal samples in AR patient.

The inhibition by GLT to HMGB1 has shown to reduce AR intensity [18,19]. However, the specific signaling pathways have not been identified. Here, we investigated the expression of HMGB1 receptors of RAGE, TLR2, and TLR4 in the nasal brushing samples. We found that the relative mRNA level of Nasal HMGB1 was significantly higher in the AR group, in addition to the higher mRNA levels of TLR2 and TLR4. However, the RAGE and TLR3 (as a TLR control) mRNA levels were not different between the AR and control groups.

Interestingly, dust mite allergies AR mice model indicated that the HMGB1-RAGE axis mediates allergic airway sensitization and airway inflammation [18]. TLR4 signaling has been suggested to participate in allergic diseases. TLR4 expression at both the mRNA and protein levels were significantly upregulated in the nasal mucosa of the patients, with allergic rhinitis [24]. The polymorphisms of TLR2 and TLR4 were significantly associated with the disease severity of asthma and allergic rhinitis [25]. Therefore, we speculated the HMGB1-RAGE axis and the HMGB1-TLR4 axis might be alternatively activated in mice and human.

It is not well recognized about the detailed regulation by HMGB1/TLRs on AR-related proinflammatory ILs. Sporadic reports imply that the upregulated TLR4 mediates the augmentation of nasal cytokine release in allergic rhinitis [26]. The TLR4 dependence was also observed in the Short Ragweed (SRW) pollen-induced allergic rhinitis and in the increased production of Th2 cytokine [27]. Our further results demonstrated the positive correlation between ILs and HMGB1/TLRs. Firstly, The R^2 value for clinical characteristics, such as age, weight or height, and ILs/HMGB1 signaling biomarkers were less than the threshold of 0.25. A high correlation of HMGB1 was found positively with IL-4, IL-5, IL-13, or IL-17A, whereas negatively with IL-10. Interestingly, the correlation of HMGB1 receptors was not seen as high as which of HMGB1 with any one of the ILs mentioned above. In particular, RAGE did not correlate with any of ILs, with HMGB1. However, a correlation of TLR4 was found positively with IL-4, IL-5, IL-13, IL-17A or IgE, or negatively with IL-10, also undoubtedly with HMGB1. Also, the linear regression model also demonstrated a significant association of nasal ILs with the nasal brushing HMGB1/TLR4 axis. Therefore, our study confirmed the involvement of HMGB1/TLR4 pathway in the IL upregulation in AR patients. However, the detailed mechanism underlining such process needs further identification.

In conclusion, high mRNA levels of HMGB1 and TLR4 in the nasal brushing samples, correlate positively with IL-4, -5, -13 or -17A, whereas negatively with IL-10 in the nasal lavage fluid samples in AR patients. HMGB1/TLR4 signaling pathway might be well responsive targets as immunotherapy for allergic rhinitis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The present study was supported by the grant from the Natural Science Foundation of China (NSFC, 81600787) and the grant from Jilin Province Science and Technology Institute (20170520028JH).

References

- [1] E.O. Meltzer, D.A. Bukstein, The economic impact of allergic rhinitis and current guidelines for treatment, *Ann. Allergy Asthma Immunol.* 106 (2011) S12–S16.
- [2] D.I. Bernstein, G. Schwartz, J.A. Bernstein, Allergic rhinitis: mechanisms and treatment, *Immunol. Allergy Clin. North Am.* 36 (2016) 261–278.
- [3] J. Bousquet, N. Khaltaev, A.A. Cruz, et al., Allergic rhinitis and its impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2) LEN and AllerGen), *Allergy* 63 (Suppl 86) (2008) 8–160.
- [4] S. Gaugris, V. Sazonov-Kocevar, M. Thomas, Burden of concomitant allergic rhinitis in adults with asthma, *J. Asthma* 43 (2006) 1–7.
- [5] G. Ciprandi, I. Cirillo, The lower airway pathology of rhinitis, *J. Allergy Clin. Immunol.* 118 (2006) 1105–1109.
- [6] A.M. Watts, A.W. Cripps, N.P. West, et al., Modulation of allergic inflammation in the nasal mucosa of allergic rhinitis sufferers with topical pharmaceutical agents, *Front. Pharmacol.* 10 (2019) 294.
- [7] A.V. Chary, R. Hemalatha, M.V. Murali, et al., Association of T-regulatory cells and CD23/CD21 expression with vitamin D in children with asthma, *Ann. Allergy Asthma Immunol.* 116 (2016) 447–454.
- [8] W. Lou, C. Wang, Y. Wang, et al., Responses of CD4(+) CD25(+) Foxp3(+) and IL-10-secreting type I T regulatory cells to cluster-specific immunotherapy for allergic rhinitis in children, *Pediatr. Allergy Immunol.* 23 (2012) 140–149.
- [9] Q. Tang, R.L. Hendricks, Interferon gamma regulates platelet endothelial cell adhesion molecule 1 expression and neutrophil infiltration into herpes simplex virus-infected mouse corneas, *J. Exp. Med.* 184 (1996) 1435–1447.
- [10] A. Kessel, T. Haj, R. Peri, et al., Human CD19(+)CD25(high) B regulatory cells suppress proliferation of CD4(+) T cells and enhance Foxp3 and CTLA-4 expression in T-regulatory cells, *Autoimmun. Rev.* 11 (2012) 670–677.
- [11] N.A. Carter, R. Vasconcellos, E.C. Rosser, et al., Mice lacking endogenous IL-10-producing regulatory B cells develop exacerbated disease and present with an increased frequency of Th1/Th17 but a decrease in regulatory T cells, *J. Immunol.* 186 (2011) 5569–5579.
- [12] C.P. Jones, L.G. Gregory, B. Causton, et al., Activin A and TGF-beta promote T(H)9 cell-mediated pulmonary allergic pathology, *J. Allergy Clin. Immunol.* 129 (2012) 1000–1010.
- [13] H.H. Erlandsson, U. Andersson, Mini-review: the nuclear protein HMGB1 as a proinflammatory mediator, *Eur. J. Immunol.* 34 (2004) 1503–1512.
- [14] A.H. Wu, L. He, W. Long, et al., Novel Mechanisms of Herbal Therapies for Inhibiting HMGB1 Secretion or Action, *Evid Based Compl. Alternat. Med.* 2015 (2015) 456305.
- [15] B. Lu, C. Wang, M. Wang, et al., Molecular mechanism and therapeutic modulation of high mobility group box 1 release and action: an updated review, *Expert. Rev. Clin. Immunol.* 10 (2014) 713–727.
- [16] H.E. Harris, U. Andersson, D.S. Pisetsky, HMGB1: a multifunctional alarmin driving autoimmune and inflammatory disease, *Nat. Rev. Rheumatol.* 8 (2012) 195–202.
- [17] L. Cavone, C. Cuppari, S. Manti, et al., Increase in the level of proinflammatory cytokine HMGB1 in nasal fluids of patients with rhinitis and its sequestration by glycyrrhizin induces eosinophil cell death, *Clin. Exp. Otorhinolaryngol.* 8 (2015) 123–128.
- [18] M.A. Ullah, Z. Loh, W.J. Gan, et al., Receptor for advanced glycation end products and its ligand high-mobility group box-1 mediate allergic airway sensitization and airway inflammation, *J. Allergy Clin. Immunol.* 134 (2014) 440–450.
- [19] Y. Liang, C. Hou, J. Kong, et al., HMGB1 binding to receptor for advanced glycation end products enhances inflammatory responses of human bronchial epithelial cells by activating p38 MAPK and ERK1/2, *Mol. Cell Biochem.* 405 (2015) 63–71.
- [20] S. Takeno, H. Yoshimura, K. Kubota, et al., Comparison of nasal nitric oxide levels between the inferior turbinate surface and the middle meatus in patients with symptomatic allergic rhinitis, *Allergol. Int.* 63 (2014) 475–483.
- [21] 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.
- [22] M.C. Lim, R.M. Taylor, R.M. Naclerio, The histology of allergic rhinitis and its comparison to cellular changes in nasal lavage, *Am. J. Respir. Crit. Care Med.* 151 (1995) 136–144.
- [23] M. Paplinska, J. Hermanowicz-Salamon, P. Nejman-Gryz, et al., Expression of eosinophils in the material from nasal brushing in asthma, allergic rhinitis and COPD patients, *Cytokine* 60 (2012) 393–399.
- [24] M. Fransson, M. Adner, J. Erjefalt, et al., Up-regulation of Toll-like receptors 2, 3 and 4 in allergic rhinitis, *Respir. Res.* 6 (2005) 100.

- [25] Y. Jin, S. Qiu, N. Shao, et al., Association of toll-like receptor gene polymorphisms and its interaction with HPV infection in determining the susceptibility of cervical cancer in Chinese Han population, *Mamm. Genome*. 28 (2017) 213–219.
- [26] A.K. Ekman, R. Virtala, M. Fransson, et al., Systemic up-regulation of TLR4 causes lipopolysaccharide-induced augmentation of nasal cytokine release in allergic rhinitis, *Int. Arch. Allergy Immunol.* 159 (2012) 6–14.
- [27] D.Q. Li, L. Zhang, S.C. Pflugfelder, et al., Short ragweed pollen triggers allergic inflammation through Toll-like receptor 4-dependent thymic stromal lymphopoietin/OX40 ligand/OX40 signaling pathways, *J. Allergy Clin. Immunol.* 128 (2011) 1318–1325.