

ANTIDEPRESSANT DRUG, DESIPRAMINE, ALLEVIATES ALLERGIC RHINITIS BY REGULATING T_{reg} AND T_H17 CELLS

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Received July 2, 2012 – Accepted January 29, 2013

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Allergic rhinitis (AR) is characterized by IgE-mediated immediate hypersensitivity and usually progresses to chronic nasal inflammation, with depression as one of its comorbidities. The importance of treating the depression in AR patients has been increasingly recognized. Desipramine is a representative of tricyclic-antidepressant agents. In the present study we investigate whether desipramine has therapeutic effects on AR inflammation. BALB/C mice were sensitized by intraperitoneal injection of ovalbumin (OVA), followed by repeated challenge with OVA intranasally. Desipramine was administered orally to treat the mice. The nasal symptoms (sneezing, nasal scratching *etc.*) of AR were evaluated to determine the severity of AR. Cytokines in the nasal lavage fluid (NALF), including interferon- γ (IFN- γ), interleukin 4 (IL-4) and serum OVA-specific immunoglobulin E (IgE) antibody were measured by ELISA. The regulatory T cells (T_{reg}) and T helper cells 17 (T_H17) were quantified by flow cytometric analysis. As a result, the repeated oral administration of desipramine attenuated the nasal symptoms (sneezing and nasal rubbing) in AR mice. Desipramine also suppressed the serum OVA-specific IgE and IL-4 levels, but had no effect on IFN- γ level. Moreover, desipramine treatment up regulated $CD4^+CD25^+Foxp3^+T_{reg}$ cells, which were found down-regulated in established AR mice. Meanwhile, desipramine administration attenuated $CD4^+IL-17^+T_H17$ cells, which were significantly increased in AR mice. These results suggest that the antidepressant drug, desipramine, also has anti-allergic action, which was possibly achieved by reducing allergen-specific IgE and T_H2 cytokine production and maintaining a balance between T_{reg} and T_H17 cells. Thus, this study provide the first evidence that desipramine may be utilized to treat allergic diseases, especially for those allergic patients with depression or depression patients with allergy.

Allergic rhinitis (AR) is described as an allergic inflammation of nasal mucosa and occurs when an allergen, such as pollen or dust, is inhaled by an individual with a sensitized immune system (1). According to statistics, there are around 10–25%

of the population affected by this common disease, most of whom are teenagers (1).

AR is caused by IgE-mediated immediate hypersensitivity in nasal mucosa, which is characterized by pruritus, sneezing, rhinorrhea, and nasal obstruction

Key words: allergic rhinitis, desipramine, T_{reg} , T_H17

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(1). When atopic individuals are exposed to allergens, IgE-mediated release of inflammatory mediators or cytokines from various immune cells actively contributes to the chronic nasal inflammation (2, 3). Although the pathogenesis of AR is not fully understood, an imbalance between allergen-specific T_H1 and T_H2 cells is believed to play an essential role (4, 5). Recently, the regulatory T cells (T_{reg}) and T helper 17 cells (T_H17) cells have also emerged to be important role players in allergic diseases (6, 7).

In addition to sneezing, nasal congestion, nasal discharge or other symptoms, AR causes various complications or comorbidities, including eczema, asthma and depression *etc* (8). A recent survey showed that young patients with AR have higher incidence of anxiety, depression and sleep disorders than general population and extreme reports showed that allergy may even become a risk factor of suicide (9). The importance of antidepressant therapy in AR has been increasingly recognized (8).

Desipramine, a tricyclic antidepressant drug, is pharmacologically characterized as a selective norepinephrine or serotonin reuptake inhibitor and has been classically used to treat depression, chronic pain, and other neurological or psychiatric disorders (10, 11). Enzyme kinetics study also showed that desipramine was a functional inhibitor of acid sphingomyelinase, the lysosomal enzyme responsible for the hydrolysis of sphingomyelin into ceramide and phosphocholine (12, 13). According to recent advances in pathogenesis of depression and pharmacology of antidepressants, antidepressant drugs could function at least in part through weakening the action of inflammatory cytokines (14, 15). Although the antidepressant effect of desipramine has been well documented, however, whether this antidepressant drug could alleviate the allergic symptoms of AR is unknown. In the present study, we investigated the pharmacological effects of desipramine on AR models in mice. To further elucidate the underlying pharmacological mechanisms, the changes of allergen-specific immunoglobulin E (IgE) antibody, cytokines (IL-4, IFN- γ), T_{reg} and T_H17 cells were also determined.

MATERIALS AND METHODS

Animals

Six-week-old male BALB/c mice were purchased

from laboratory animal center (medical college, HUST, CHINA, No.8836). Mice were kept in an animal room, with temperature 22 ~ 26°C, humidity 40% ~ 70%, light-dark cycle 12h/12h, throughout the experimental course. The study was reviewed and approved by the medical ethical committee of the author's institution.

Murine AR model and treatment

Mice were randomly divided into five groups (control group; AR group; Low-dose desipramine group; middle-dose desipramine group; high-dose desipramine group;) and each group consisted of 6 mice. The murine AR model was established as described previously (6). All mice except those in the control group were sensitized by an intraperitoneal injection of Ovalbumin (OVA, 1 μ g, Grade V, Sigma) in 200 μ l of saline containing aluminium hydroxide (Sigma, 100 μ g) as an adjuvant on day 0, 5, 14, and 21. After sensitization, the animals were challenged by instilling OVA in physiological saline (20mg/ml, 10 μ l) into the bilateral nasal cavities three times weekly during the following five weeks. The control group received physiological saline instead of the allergen. Thirty min before intranasal OVA challenge, mice received corresponding medication except for control group and AR group that received saline. The low, middle and high dose of desipramine (Sigma, USA) was 3mg/kg, 10mg/kg, 30mg/kg by intragastric injection respectively. The doses were determined in preliminary experiments.

Evaluation of allergy-related behaviors

The times of sneezing and nasal rubbing of each mouse within 30 minutes after intranasal OVA challenge were recorded by 2 independent observers weekly.

H.E. staining of mice nasal mucosa tissue

Twenty-four hours after the last challenge, the mice were sacrificed by cervical dislocation. The nasal dorsum were quickly opened, stripped of maxillary fur and cut along the lateral septum. After the nasal septum and bilateral nasal cavity were exposed, the nasal mucosas of nasal septum were collected and underwent fixation, dehydration, and embedding process. After sectioned into slices, each slice was stained with hematoxylin and eosin (H.E.) and the pathological changes were observed under microscopy.

Measurement of serum OVA-specific IgE antibody

After the mice were sacrificed, the blood samples were taken from ophthalmic veins. The serum was obtained after centrifugation (3000 rpm, 10 min at 4°C). The OVA-specific IgE in serum was measured with an ELISA kit (Chondrex, USA, Cat No. 3010) following the

manufacturer's instructions.

Measurement of IL-4, IFN- γ in nasal lavage fluid (NALF)

After the mice were sacrificed and disinfected, a tracheotomy with epidural catheter into the nasopharynx was conducted. The nasal mucosa was lavaged by 1 ml cold PBS from the choana to the anterior nostril and NALF was collected. After centrifugation at 3000 rpm, 10 min, 4°C, the supernatant was collected. The levels of IL-4, IFN- γ in the NALF were measured with the ELISA kits (R&D Systems, USA, Cat No. 0356; 1327) according to the manufacturer's instruction.

The ratio of CD4⁺CD25⁺Foxp3⁺cells/CD4⁺ cells, and CD4⁺IL-17⁺cells /CD4⁺cells in the spleen cells

T_{reg}s are now recognized as an important component of the immune system that suppresses immune responses. T_{reg}s come in many flavors with the most well known being those that express CD4, CD25, and Foxp3 (CD4⁺CD25⁺ regulatory T cells, or "T_{reg}s"). T helper 17 cells (T_h17) are a subset of T helper cells producing interleukin 17 (IL-17) and are considered developmentally distinct from T_h1 and T_h2 cells (16). The ratio of CD4⁺CD25⁺Foxp3⁺cells/CD4⁺ cells and ratio of CD4⁺IL-17⁺ /CD4⁺cells were measured as described previously (17). The spleen was removed in sterile condition and was made into single-cell suspension which was adjusted to a concentration of 1x10⁶/ml in RPMI 1640 medium (Gibco, USA). The CD4⁺CD25⁺Foxp3⁺cells in the spleen single-cell suspension were assayed using mouse regulatory T cell staining kit (EB, USA, cat No. 88-811), according to the manufacturer's instructions. In brief, the cell surface were first stained with anti-mouse CD4-FITC and anti-mouse CD25-APC antibodies, then the cells were permeabilized and further stained with anti-Foxp3 antibody. The concentrations of CD4⁺CD25⁺Foxp3⁺cells were determined by flow cytometry (BD Bioscience). To determine the ratio of CD4⁺IL-17⁺cells /CD4⁺cells, the stimulants, phorbol 12-myristate 13-acetate (PMA) (Enzo, USA), ionomycin (Enzo, USA), brefeldin A (BFA) (Enzo, USA), were added into the spleen single-cell suspension and the cells were incubated in a CO₂ incubator at 37°C for 4 h. After the spleen single-cell suspensions were stained with FITC-CD4⁺ antibodies (EB, USA, Cat No11-0042), the cells were then permeabilized and further stained with PE-labeled IL-17A antibodies (EB, USA, Cat No17-7177-81). The number of CD4⁺IL-17⁺ T cells was determined by flow cytometric a

Statistical analysis

All the data were expressed as mean $\pm$ SE. Single-factor analysis of variance (ANOVA) in the Dunnett *t*-test for multiple comparisons between groups was used to conduct the statistics. *P* < 0.05, the difference was

considered statistically significant.

RESULTS

Desipramine attenuated the allergy-related behaviors in sensitized mice

As shown in Fig. 1, compared to control, OVA sensitization significantly induced the allergy-related behaviors (sneezing and nasal rubbing) in mice of AR group. Repeated oral administration of desipramine reduced the frequencies of sneezing and nasal rubbing induced by OVA sensitization, especially in middle-dose and high-dose desipramine group. From the 35th day (1 week after desipramine treatment) in high-dose desipramine group and from the 49th day (3 week after desipramine treatment) in middle-dose desipramine group, the frequencies of sneezing and nasal rubbing began to decrease statistically significantly.

The pathological changes in mice nasal mucosa

As shown in Fig. 2, in the AR group (B), impairment of the cilia and eosinophil infiltration

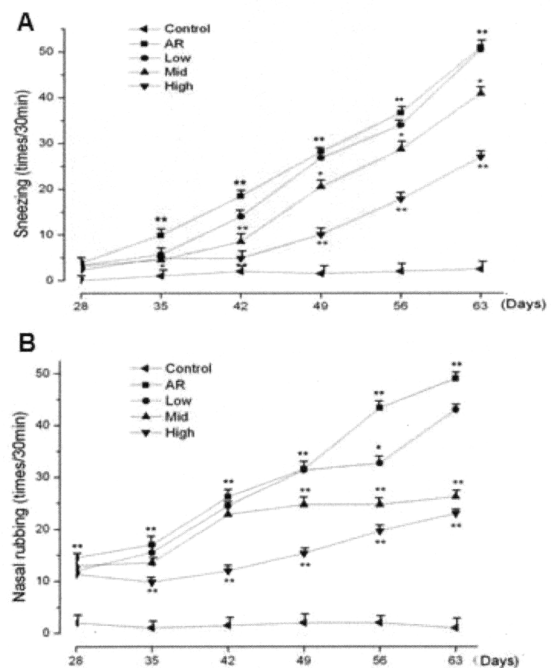


Fig. 1. Effect of desipramine on sneezing (A) and nasal rubbing (B) behaviors in mice. *, *P* < 0.05, vs AR group; **, *P* < 0.01 vs AR group. *n* = 6 mice for each group.

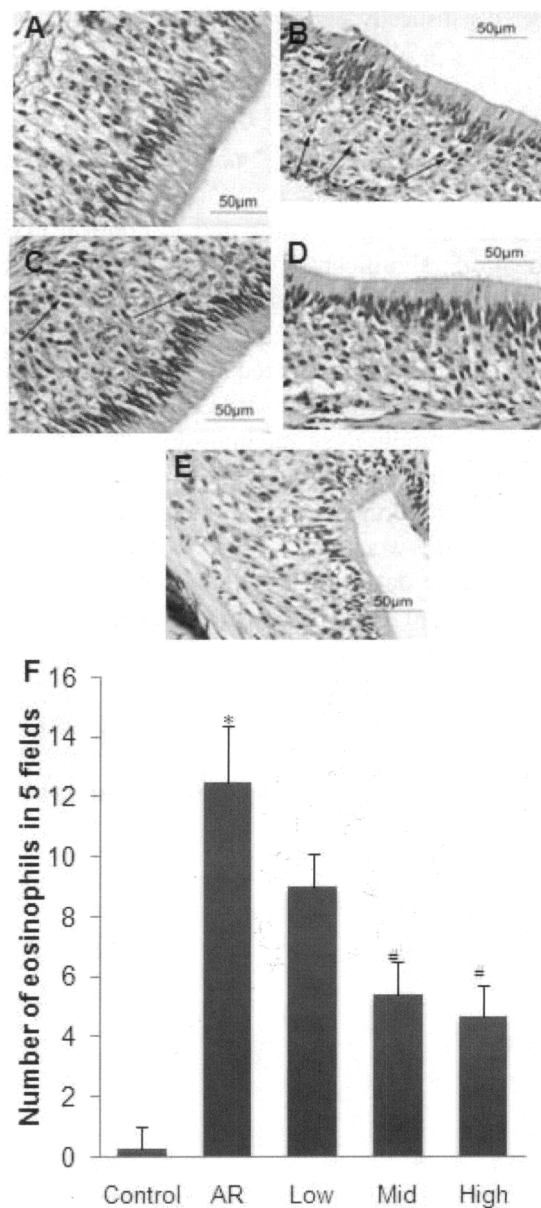


Fig. 2. Histopathologic changes of mice nasal mucosa. **A)** control group; **B)** AR group; **C)** low-dose desipramine group; **D)** middle-dose desipramine group; **E)** high-dose desipramine group. **F,** quantitative analysis of eosinophils in nasal mucosa. *, $P < 0.01$ vs. control. #, $P < 0.05$, vs. AR group. $n = 6$ mice/group.

(indicated by arrowhead and summarized in Fig. 2F) were observed in the nasal mucosa, which were largely absent in control group (A). The alterations reflecting the inflammatory responses attenuated in the desipramine-treated groups (C, D, E) as

compared to those in the AR group.

Serum OVA-Specific IgE

IgE represents a relatively specific marker of allergy. As depicted in Fig. 3, in the control group, almost no OVA-specific IgE was detected. However, in the AR group, serum OVA-specific IgE level was significantly increased (237.91 ± 7.76 ng/mL). Desipramine treatment dose-dependently reversed the OVA-induced increase of the specific IgE levels, with a value of 162.92 ± 7.70 ng/mL in the low dose group, 94.01 ± 6.29 ng/mL in middle dose group and 21.41 ± 3.62 ng/mL in high dose group.

Cytokines in NALF

As shown in Fig. 4A, IFN- γ level in the AR group increased significantly (10.92 ± 0.73 pg/mL) when compared with that in the control group (3.77 ± 0.51 pg/mL). However, no significant difference was detected for IFN- γ content between AR group and low-dose desipramine group (10.5 ± 1.01 pg/mL) or middle-dose desipramine group (11.17 ± 0.65 pg/mL) or high-dose desipramine group (9.22 ± 0.2 pg/mL). As shown in Fig. 4B, IL-4 level in the NALF were significantly increased in the AR group (44.53 ± 1.26 ng/mL) as compared to that in the

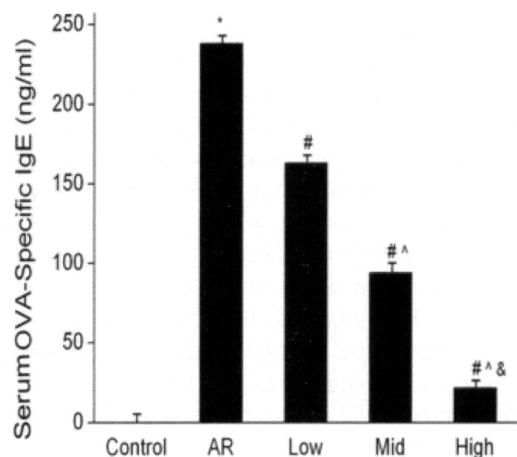


Fig. 3. OVA-specific serum IgE levels in mice. *, $P < 0.01$ vs control. #, $P < 0.01$, vs AR group. ^, $P < 0.01$, vs Low dose group. &, $P < 0.01$, vs Middle dose group. $n = 6$ mice/group.

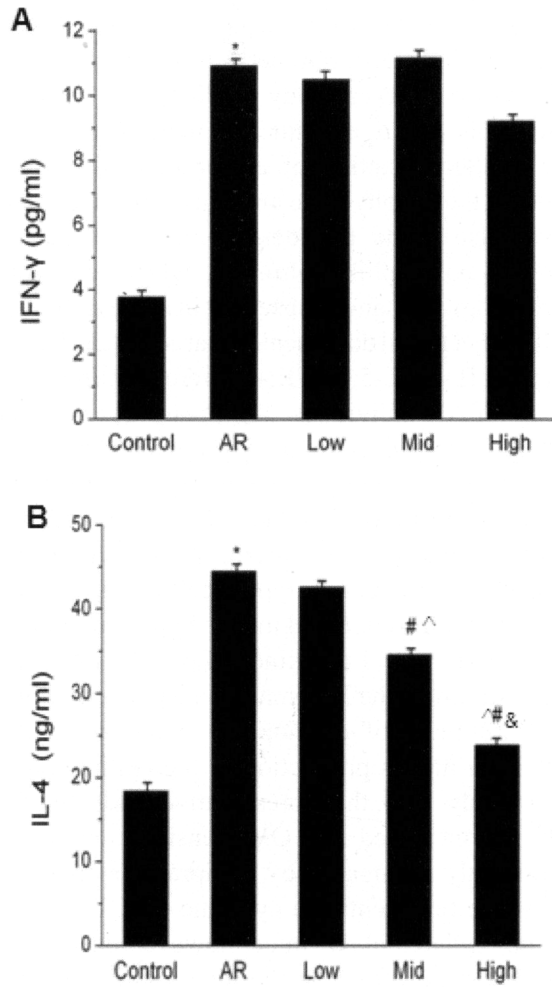


Fig. 4. Cytokine levels in the nasal lavage fluid (NALF) of mice. IFN- γ (A) and IL-4 (B) in NALF were measured by ELISA. *, $P < 0.01$, vs control. #, $P < 0.01$, vs AR group. ^, $P < 0.01$, vs Low dose group. &, $P < 0.01$, vs Middle dose group. $n = 6$ mice/group.

control group (18.33 ± 0.43 ng/mL). Middle-dose and high dose desipramine treatment significantly reversed the increase of this cytokine induced by sensitization, with IL-4 levels of 32.83 ± 0.54 ng/mL and 23.85 ± 0.29 ng/mL in both groups respectively. However, IL-4 content in the low-dose desipramine group (42.6 ± 0.94 ng/mL) showed no significant difference when compared to AR group.

The ratio of CD4⁺CD25⁺Foxp3⁺/CD4⁺T lymphocyte subset in mice spleen cells.

As shown in Fig. 5A, CD4⁺CD25⁺T subsets

in mice spleen were first gated by flow cytometry and then CD4⁺Foxp3⁺T subsets were gated (5B). CD4⁺CD25⁺Foxp3⁺/CD4⁺T lymphocyte subset were calculated by the multiply of the values obtained in the former two protocols. As summarized in Fig. 5C, flow cytometric analysis showed that the ratio of CD4⁺CD25⁺Foxp3⁺/CD4⁺T lymphocyte subset in mice spleen cells was decreased significantly in the AR group ($7.83\% \pm 0.46\%$) when compared with that in the control group ($14.86\% \pm 0.41\%$). High-dose desipramine treatment partly reversed the decrease of this ratio ($8.96\% \pm 0.13\%$). Although low-dose desipramine ($8.11\% \pm 0.25\%$) and middle-dose desipramine ($8.62\% \pm 0.36\%$) treatment also showed some reversing effects in this respect, however, it is not statistically significant.

The ratio of CD4⁺IL-17⁺/CD4⁺T lymphocyte subset in mice spleen cells.

Figs. 6A and 6B present the representative graphs of the flow cytometric analysis of CD4⁺IL-17⁺

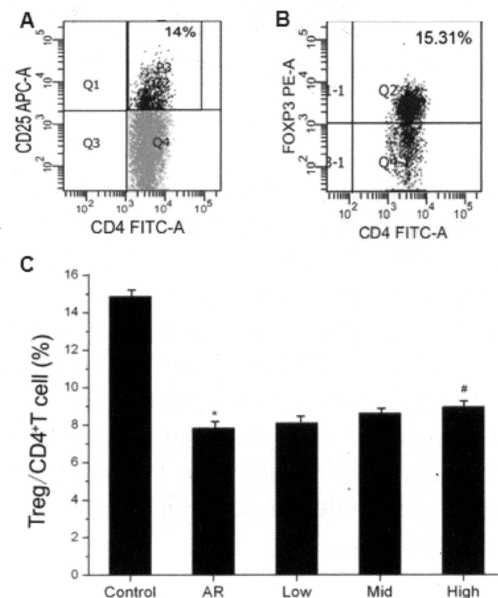


Fig. 5. Flow cytometric analysis of CD4⁺CD25⁺Foxp3⁺T lymphocyte subset from mice spleen. A) Representative graph of CD4⁺CD25⁺T subsets were gated by flow cytometry; B) representative graph of CD4⁺Foxp3⁺T subsets were gated by flow cytometry; C) summary of the ratio of CD4⁺CD25⁺Foxp3⁺/CD4⁺T lymphocyte subset in mice spleen cells. *, $P < 0.01$, vs control; #, $P < 0.05$, vs AR group.

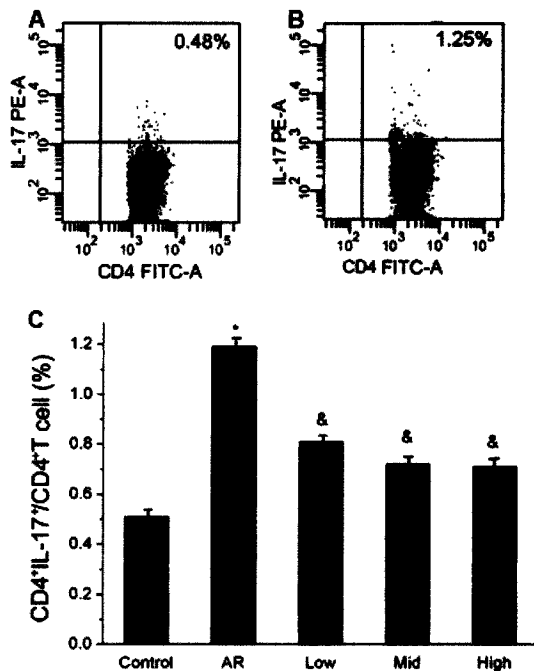


Fig. 6. Representative graphs of flow cytometric analysis of CD4⁺IL-17⁺ T lymphocyte subset in control (A) and AR (B) group respectively. C. summary of the ratio of CD4⁺IL-17⁺ / CD4⁺ T lymphocyte subset in mice spleen cells. *, $P < 0.01$, vs control; &, $P < 0.01$, vs AR group.

/ CD4⁺T lymphocyte subset in control and AR mice spleen cells. As summarized in Fig. 6C, the ratio of CD4⁺IL-17⁺ / CD4⁺ T lymphocyte subset in mice spleen cells increased significantly in the AR group ($1.19\% \pm 0.04\%$) when compared with that in the control group ($0.51\% \pm 0.04\%$). Desipramine treatment significantly down-regulated the allergy-induced increase of these IL-17 producing cells in all three doses ($0.81\% \pm 0.06\%$ in low dose group, $0.72\% \pm 0.02\%$ in middle dose group, $0.71\% \pm 0.03\%$ in high dose group).

DISCUSSION

In the present study, we found that the antidepressant drug, desipramine, also had anti-allergic actions in mouse AR models. This was supported by evidence obtained from behavior, and histological and immunological studies. In this regard, we suggest that new therapeutic applications as treatment of AR or other allergy-related disorders

for antidepressant drugs are feasible.

In the present study, the data showed that repeated treatment with desipramine induced a decrease in OVA-specific IgE antibody production in sensitized mice. This could explain the majorities of anti-type 1 allergic action of desipramine by directly reducing the anaphylactic antibody, IgE. In addition, it was also found that desipramine decreased the grade of eosinophil infiltration, which are typical morphological changes observed in allergic diseases.

It has been well documented that activated T_H2 cells secreting IL-4, IL-5, and IL-13 were identified in the airway of AR individuals (4). IL-4 plays important roles in T_H2 cell development, growth and activation and participates in regulating the equilibrium of T_H1 / T_H2 balance (4). IL-4 induces B cell proliferation, activation and promotes the IgM, IgG-type B cells to transform into the IgE-type B cells, thus leading to eosinophil infiltration and mucus hypersecretion (4). On the contrary, T_H1 cytokines (IFN- γ , IL-12 *etc.*) are able to diminish the T_H2 immune response and block the recruitment of eosinophils to the airway and inhibit the mucus production in the epithelium (4). In this study, IL-4, the representative cytokine of T_H2 cells, was increased after OVA sensitization, but was significantly attenuated by desipramine treatment. IFN- γ , the representative cytokine of T_H1 cells was also upregulated in AR mice; however, desipramine had almost no effect on the level of IFN- γ . These results suggest that both T_H1 and T_H2 cytokines are set to a reinforcement state in AR mice, but desipramine appears to exert its therapeutic effect by selectively suppressing the actions of T_H2 cells.

T_{reg}, the new CD4⁺ T lymphocytes subset, is reported to effectively control the immune responses to achieve moderate immune tolerance by discriminating between self and non-self (18). CD4⁺CD25⁺T_{reg} has been shown to exert a suppressor action on the immune system by cell-to-cell contact with the cell being suppressed (18). A recent study demonstrated that in children with allergic airway disease, the number of T_{reg} cells decreased significantly (19). CD4⁺T lymphocytes also include T_H17 cell subset, which produces IL-17 dependent on IL-23 (16). The function of IL-17 is to promote inflammation, increasing the local production of chemokines and stimulating the production of IL-6 and prostaglandin E2 to amplify the local

inflammation. Ba et al had shown that in the nasal mucosa of AR patients, T_h17 cells were significantly upregulated, indicating the possible involvement of T_h17 cells in the development of AR (20). Recently, increasing studies have reported that IL-17 in the lungs, sputum, bronchoalveolar lavage fluid of asthma patients were also significantly upregulated and IL-17 levels in the serum were closely associated with the severity. In this study, $CD4^+CD25^+T_{reg}$ cells in the AR mice were found significantly decreased, however, $CD4^+IL-17^+$ cells in the AR mice were found increased. These results were consistent with many previous reports related with allergic diseases (22, 23), suggesting that a potential immune balance between $CD4^+CD25^+T_{reg}$ cells and $CD4^+IL-17^+$ cells was maintained in the healthy population, but turned into a status of immune imbalance in allergic patients. It appears that the ratio of T_{reg}/T_h17 is tending to decrease in these allergic diseases. In the present study, when desipramine was used to treat AR, the imbalance of T_{reg}/T_h17 in AR mice was partly reversed, which could at least in part explain the therapeutic effects of desipramine on the allergic diseases.

Recently, the therapeutic effects of desipramine in the treatment of inflammation have been reported in many studies. In the LPS-induced septic shock model, preventive treatment with desipramine reduced tumor necrosis factor α (TNF- α) production and produced almost equally protective effects as the glucocorticoid, prednisolone (24). A previous study from our laboratory also demonstrated that desipramine was able to inhibit TNF- α -induced reactive oxygen species (ROS) production and nuclear kappa B (NF- κ B) signaling in airway epithelial cells by inhibiting the acid sphingomyelinase (25). Administration of the tricyclic antidepressant desipramine in rats has been shown to result in a decrease of neuron-derived TNF- α (26). It has also been reported that antidepressants are able to decrease peripheral inflammation (14, 27). Becker et al reported that desipramine treatment normalized the inflammation in lung cystic fibrosis (28). The mechanisms underlying the anti-inflammatory effect of desipramine are not fully elucidated. Recently, lipid rafts are emerging to play essential roles in transmembrane signal transduction. Many receptors or co-stimulatory molecules are able to activate

acidic sphingomyelinase, which can hydrolyze the sphingomyelin in the cell membrane and release large amounts of ceramides (29). Ceramides are able to integrate the small non-active microdomains into the large active ceramide-enriched platforms (29). The macromolecular proteins and molecules being recruited to the platform are intended to implement and amplify the signals (29). Desipramine could suppress the small lipid rafts to fuse into large platforms by decreasing the production of ceramide as a result of inhibiting the activity of acidic sphingomyelinase, thus leading to some negative effects on the initiation of downstream signal transduction (30); Moreover, desipramine also inhibits the infiltration of various inflammatory cells and the production of inflammatory mediators (26). It is possible that the combined effects of desipramine lead to the relief of AR inflammation by this drug.

In summary, in addition to the well-known T_h1/T_h2 imbalance in the pathogenesis of AR, a T_{reg}/T_h17 imbalance may also exist in this disease. This may widen the understanding of the immunological mechanisms in AR and implicate new strategies for treating this disease. Since there is increasing prevalence of depression in patients with AR, asthma or other chronic inflammatory diseases, the anti-inflammatory effects of antidepressant, desipramine, should be considered, especially in treating allergic patients accompanied by depression or depressive patients with inflammatory co-morbidities. Hopefully, it will bring relative more complete relief of both physical and psychological problems in these patients as compared to those drugs targeted on allergic inflammation only, thus may improve the life qualities of affected patients in a great deal. In clinic, physicians tend to underestimate the psychological problems in patients with AR. If more attention is paid on this issue, desipramine and its similar analogues might become good choices for the treatment of these diseases.

ACKNOWLEDGEMENTS

This study was supported by grants from the National Natural Science Foundation of China (30870997, 81072634), the Ministry of Education of China (NCET-10-0409) and the authors' institution (HUST2011TS069).

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