The most prevalent and well-investigated indoor perennial allergen source is House Dust Mites (HDM). HDM major allergen, Der p 2, can induce proinflammatory cytokine production which is conducted by NLRP3 inflammasome activation. Furthermore, Der p 2 could upregulate expression of autoantigen TRIM-21 and autoantibody anti-PGK-1 production in B cells and PBMCs derived from SLE patients. Systemic lupus erythematosus (SLE) is a chronic, remitting and relapsing multisystem autoimmune disease. Many different autoantibodies which respond to autoantigens are found in patients with SLE. Many SLE patients also have allergies, and immunological events triggering the onset and progression of the clinical manifestations of SLE by allergens have been reported. Newly discovered cell penetration peptides derived from human eosinophil cationic proteins (CPPecp) could downregulate HDM allergen-induced pro-inflammatory cytokine production by increasing IFN-α expression. According to these findings, environmental allergen might play a role in the pathogenesis of autoimmune disease, and the modulation of inflammasome activation caused by allergen might be of importance in the prevention of the development of autoimmune disease. Journal of Nature and Science, 1(7):e126, 2015.

House dust mite | Innate immunity | Proinflammatory cytokine | Inflammation

**Introduction**

Allergic diseases affect around 15–35% of populations worldwide [1]. The major mechanism of allergic diseases is the immediate-type responses triggered by allergen binding with IgE antibodies on the surface of mast cells and basophil, followed by the release of cytokines and inflammatory mediators, which induce symptoms within minutes of allergen contact.

The most studied mode of basophil activation begins with FcεRIα- and IgE-mediated crosslinking [2]. Activated basophils produce histamines and leukotrienes in response to IgE-mediated activation [3]. The release of these inflammatory substances results in the induction of smooth muscle contraction, thereby contributing to bronchial asthma. Furthermore, basophils could respond directly to protease activity, such as the house dust mite (HDM) major allergen Der p 1, which is capable of increasing the production of Th2-related cytokines such as IL-4, IL-5, and IL-13 from human basophil cell lines [4].

Another allergy phenotype is commonly associated with Th2 response [5]. Th2 cells play a critical role in orchestrating the inflammatory response in allergy through their secretion of IL-4 and IL-13-mediating B cell activation and IgE synthesis [6, 7]. Th2 response is dependent on dendritic cells (DCs) which are capable of producing specific cytokines, such as IL-10 and IL-33, to facilitate skewing toward Th2 differentiation [8-10]. Allergic responses could be activated by various kinds of allergens with different biological functions, including HDM, pollen, cockroach, and fungal spores [11]. These allergens can be characterized based on whether they display enzymatic activity. It has been recommended that allergens be divided into class 1 allergens, which possess enzymatic activity (Der p 1, Der p 3), and class II allergens, which do not show enzymatic activity (Der p 2) [12].

**HDM allergy**

HDM allergy is the most common Aeroallergen. It has a well-established causal role in patients with allergic diseases, such as atopic dermatitis (AD), allergic rhinitis (AR), and allergic asthma (AA) [13, 14]. HDM could be identified in dust and stuffing such as mattresses, pillows, stuffed animals, and bedding [15]. The most common species are Dermatophagoides pteronyssinus (Der p), Dermatophagoides farina (Der f) and Blomia tropicalis (BT). In Taiwan, the climate is warm and humid which could be a major factor for promoting dust mite growth. The prevalence of HDM allergy in children and adolescents with asthma exceeds 50% [16].

There are more than twenty different allergens of HDM which could be identified by specific IgE derived from HDM-sensitized patients [17]. Although the biological functions of HDM allergens have not been fully clarified, these allergenic proteins can be categorized into four main families: proteases, proteins displaying affinities for lipids, non-proteolytic enzymes, and non-enzymatic components [18]. Der p 1 (group 1 HDM allergen) is considered a cysteine proteinase, and Der p 3,6,9 (groups 3, 6, 9 HDM allergen) are trypsin-like, chymotrypsin-like, and collagenolytic-like serine proteinases, respectively. These protease allergens could play an important role in digestion for the mite as they were detected in the gut and feces of HDM [19]. According to their structural or sequence homologies, Der p 2, 5, 7, 13, 14, and 21 HDM allergen could be characterized as fatty acid/lipid binding proteins. However, functions of these allergens in the mite require further investigation [20]. Other HDM allergens show enzymatic activities, such as, Der p 4, 8, and 20 (group 4, 8, and 20 HDM allergens) are amylases, glutathione-S-transferases, and arginine kinases, respectively. In addition, Der p 12, 15, and 18 (groups 12, 15, and 18 HDM allergen) display homologies with chitinases [21-24]. Der p 10 and 11 (group 10 and 11 HDM allergen) are composed of the muscle-derived proteins tropomyosin and paramyosin, respectively [25, 26]. Der p 16 and 17 (groups 16 and 17 HDM allergen) have been identified as gelsolin-like and EF-hand Ca 2+-binding proteins [27, 28].

**Der p 2 could enhance NF-κB activity by activating TLR4 signal transduction**

Although more than twenty groups of HDM allergens have been identified, the most important major allergen is Der p 2. Der p 2 and its specific IgE in the sera are highly correlated with allergic hypersensitivity in patients with asthma, atopic dermatitis, and allergic rhinitis. It has been estimated that 79.2% of patients with asthma, wheezing and/or rhinitis have IgE antibodies to Der p 2 [29]. Recently, the Der p 2 allergen was found to show structural homology with MD-2, suggesting that Der p 2 tends to cause targeted immune responses because of its autoadjuvant properties.
The structure of Der p 2 provides a useful tool in the design of recombinant immunotherapeutics for the group-2 allergens[30]. Der p 2 has been demonstrated to be capable of triggering human B cell activation and TLR4 induction. Der p 2 markedly induced the expressions of several key cytokines, including IL-1β, CXCL10, IL-8, and TNF-α in B cells. Der-p2 could also enhance NF-kB activity, indicating that Der-p2 may exert its influence through NF-kB activation to induce the production of proinflammatory cytokines. Moreover, Der p 2 specifically upregulated MKP-1 expression and activity in human B cells, which in turn resulted in p38/MAPK dephosphorylation, triggering TLR4 induction[31]. mRNA expression of CXCL5, IL-1β, IL-6, and IL-8 were upregulated after Der p 2 stimulation for six hours (fig.1) [33]. Furthermore, we also investigated the correlation between Der p 2 and proinflammatory cytokine production by culturing PBMCs derived from six HDM-allergic subjects cultured with Der p 2 (1.5ug/ml) or LPS (500ng/ml) for six hours. The results showed that IL-1β, IL-6, and IL-8 were significantly upregulated after Der p 2 stimulation (p<0.05; Fig.2) [34]. The purpose of these cytokines and chemokines secreted by innate immune cells is to upregulate T-cell proliferation and cytokine production, which consequently activates the adaptive immune response [35].

Receptors of innate immune response
There is a set of receptors classified as PRRs which is responsible for the innate immune response by identifying stimuli such as PAMPs and damage-associated molecular pattern molecules (DAMPs). There are many types of immune cells expressing PRRs, such as macrophages, monocytes, and airway epithelium cells. There are four main classes of PRRs which are classified by distinct genetic and functional characteristics, which consist of the following: membrane-bound toll-like receptors (TLRs), nucleotide binding and oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), and C-type lectin receptors (CLRs) [32]. PAMPs could be detected by TLRs and CLRs on the cell surface and in intracellular compartments, and RLRs and NLRs sense PAMPs in intracellular compartments. Unlike other PRRs, NLRs could also recognize DAMPs in intracellular compartments [36]. In recent years, NLRs have been investigated with respect to their involvement in allergic inflammation. NLRs are generally assembled at three separate domains. They have a variable effectors domain at the amino terminus which is either a pyrin domain (PYD), a caspase activation and recruitment domain, (CARD) or a baculovirus inhibitor of apoptosis protein repeat (BIR). The effectors domains mediate homotypic protein–protein interactions. The carboxy terminus of NLR proteins contains a ligand-binding leucine-rich repeat domain (LRR) which is responsible for ligand sensing and autoregulation [36]. A central nucleotide-binding and oligomerization domain (NOD) is responsible for delivering the signal to the dNTPase activity and self-oligomerization [37]. These receptors are further subdivided into three distinct groups, according to their functions and the similarities in domain structures, NOD family genes (NOD1-2, NOD3/NLRC3, NLRGS/NOD4, NLRX1/NOD5, CIITA), the NLRP family (NLRP1-14, previously called NALPs), and the IPAF family (NAIP, NLRC4 previously called IPAF). However, among these three groups, a significant subset of NLRs is believed to activate the inflammasome, a multi-protein complex that self-oligomerizes and assembles upon cellular infection or tissue damage to either induce an inflammatory response or cell death. It has been reported that NLRP1, NLRP3, and NLRC4 have a well-characterized physiological inflammasome function [38].

Inflammasome activation is correlated with proinflammatory cytokine production
The inflammasomes are a group of protein complexes that sense several inflammation-inducing stimuli such as virus infection, allergen, and cellular damage. After detecting the stimuli, inflammasomes coordinate and consolidate these diverse signals to trigger cellular responses. Inflammasome self-oligomerization and caspase-1 auto-activation consequently induces maturation of proinflammatory cytokines such as IL-1β to modulate innate immune responses, or to trigger caspase-1-mediated cell death. It has been reported that proinflammatory cytokines expression is related to inflammasome activation [39]. The inflammasome plays an important role in allergic inflammation and innate immunity. When inflammasome is activated, caspase-1 cleaves pro-IL-1β into active IL-1β followed by the activation of diverse downstream signalling pathways and proinflammatory processes. There are four different subtypes of the inflammasome; however, the nucleotide-binding domain and leucine-rich repeat protein 3

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**Figure 1.** Der p 2 could increase the expression of TH17 related pathway genes. PBMCs derived from HDM allergic patients (n=6) were treated with Der p 2 (1.5ug/ml) for six hours. mRNA was extracted and detected by qPCR. Concentration of HDM specific IgE: Low: 0.39KU/L, Moderate: 80.1 KU/L, High: >100KU/L.

**Figure 2.** The effects of Der p 2 on inducing pro-inflammatory cytokine expression in HDM allergic patients. PBMCs derived from HDM allergic patients (n=6) were treated with Der p 2 (1.5ug/ml) for six hours; LPS (500ng/ml) was used as control. After treatment, the culture supernatant was collected and the concentration of IL-1β, IL-6, and IL-8 was measured by ELISA. Bars and error bars indicate mean and standard error of the mean (SEM), respectively. * p<0.05 compared to control group.

Proinflammatory cytokines could be upregulated by Der p 2 exposure
It has been reported that mite major allergens could activate the innate immune system. The innate immune system is the first line of defense in the human immune system with a relatively rapid response. As the innate immune system is consistent and non-specific, its response to repeated challenges will not change [32]. In HDM allergy, when the host comes into contact with an allergen, pattern recognition receptors (PRRs) of the innate immune cells recognize pathogen-associated molecular patterns (PAMPs) then cytokines and chemokines are secreted which recruit other inflammatory cells and proinflammatory cytokines to amplify the inflammatory responses. Mite major allergens could induce both innate and adaptive immune responses. In our previous study, PBMCs derived from three patients with SLE and HDM allergy were used to investigate the correlation between Der p 2 and proinflammatory cytokine production. The results showed that...
Apoptosis is activated through ligation of FAS receptors on the cell surface or passively induced through decreased essential survival signals. Apoptotic cells go through a series of different morphological changes such as cytoskeletal disruption, cell shrinkage, DNA fragmentation, and plasma membrane blebbing [48]. It has been reported that nuclear autoantigens targeted in SLE are concentrated within apoptotic blebs [49, 50].

NETosis, a specialized form of neutrophil cell death, was first described a few years ago [51]. NETosis is related to SLE because of an additional source of autoantigen production [52]. Pathogens and sterile inflammatory mediators such as monosodium urate (MSU) crystals, IL-8, IL-1β, platelet-activating factor (PAF), and TNF-α have been reported to upregulate NETosis [53]. Apoptosis is a relatively more organized and programmed phenomenon, while NETosis appears to be a faster process and less well-coordinated.

In the recent years, bystander activation of the immune system in SLE patients has been shown to be related to environmental factors such as allergens. Bystander activation means an indirect or non-specific activation of autoimmune cells caused by the inflammatory environment present during infection [54]. It can lead to enhanced processing and presentation of self-antigens, which induces the expansion or spreading of the immune response toward different self-antigens [55, 56]. In our previous study, we investigated the effect of Der p 2 on well-known autoantigen-TRIM-21 production. The results showed that the level of TRIM-21 from SLE patients with positive anti-TRIM-21 and HDM allergy was significantly upregulated after Der p 2 stimulation for 48 hours compared to that of SLE patients. (126.01% ± 11.15 versus 95.25% ± 5.89, p < 0.05) (Fig.3)[33].

Figure 3. Der p 2 could upregulate TRIM-21 production from B cells derived from SLE patients with HDM allergy. B cell lines (1x10^6 cells/ml) derived from SLE with HDM allergy (n=10) and SLE without HDM allergy (n=6) patients were treated with Der p 2 (10ug/ml) for two days. Protein level of TRIM-21 was detected by Western blotting and results were shown as percent change of control (mean±SEM). *p<0.05.

Figure 4. Der p 2 could enhance anti-PGK-1 secretion on PBMCs derived from SLE patients. PBMCs (1x10^6 cells/ml) derived from patients with SLE patients (n=9) were treated with Der p 2 (10ug/ml) for five days. Supernatant was collected and analyzed by PGK-1 ELISA. Bars and error bars indicate mean and standard error of the mean (SEM), respectively. * p<0.05.

Another feature of SLE is the production of autoantibodies against different autoantigens which were found in apoptotic and necrotic cells. Some of these autoantibodies show a linkage between

### Table 1. Prevalence of allergic diseases between the non-SLE and SLE patients

<table>
<thead>
<tr>
<th>Clinical manifestation</th>
<th>Non-SLE</th>
<th>SLE</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic disease, total</td>
<td>1545/3832 (40.3%)</td>
<td>441/958 (46.0%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Age &lt; 20 years</td>
<td>241/485 (49.7%)</td>
<td>77/131 (58.8%)</td>
<td>0.065</td>
</tr>
<tr>
<td>Age 20-40 years</td>
<td>717/1736 (41.3%)</td>
<td>198/433 (45.7%)</td>
<td>0.095</td>
</tr>
<tr>
<td>Age &gt; 40 years</td>
<td>587/1611 (36.4%)</td>
<td>166/394 (42.1%)</td>
<td>0.036</td>
</tr>
</tbody>
</table>

* Allergic diseases include asthma, allergic rhinitis and allergic dermatitis.

# The data source was Taiwan's National Health Insurance Research Database (NHIRD), which contains inpatient and ambulatory care claims. The study subjects enrolled all female SLE patients (International Classification of Diseases, 9th Revision, Clinical Modification [ICD9-CM] Code 717.1) in Taiwan from the registry of patients with catastrophic illness in 2009. The prevalence rates of allergic disease in 2009, including asthma (ICD9-CM Code: 493), allergic rhinitis (ICD9-CM Code: 477), and allergic dermatitis (ICD9-CM Code: 691), were estimated.

The association between systemic lupus erythematosus and house dust mite allergy

We analyzed the prevalence of allergic diseases in systemic lupus erythematosus (SLE) patients based on data obtained from Taiwan's National Health Insurance Research Database. The results showed that the prevalence of allergic diseases was significantly higher in SLE patients compared with that of non-SLE patients (SLE: 46.0% versus Non-SLE: 40.3%, p < 0.001). When the prevalence of allergic diseases was stratified by age group, a significantly higher prevalence rate of allergic disease was seen in SLE patients over forty years old compared with that in non-SLE patients (Table 1).

SLE is a multifactorial systemic autoimmune disorder which affects multiple organs including the skin, lung, joints, kidneys, and heart[46]. The pathogenesis of SLE is related to genes, environmental factors, and abnormalities of both the innate and the adaptive immune system. In some SLE patients, autoantibodies could target specific proteins in the cytosol or nucleus. These proteins are called autoantigens. Since these self-antigens are normally covered in the extracellular space by the nuclear membrane and the cell membrane, it is not yet known how these self-antigens are exposed or upregulated and then detected by the immune system. There are two sources of autoantigens in SLE: one is impaired clearance of apoptotic cells and the other is NETosis, a specialized form of neutrophil cell death. In our previous study, environmental factors such as HDM major allergen could also induce B cells derived from SLE patients bystander activation leading to production of autoantigens [33].

For the past two decades, apoptosis has been thought of as the most well-characterized and is related to allergic airway inflammation and asthma [40-43]. NLRP3 is a protein of 1016 amino acids transcribed from the gene *cias1*, which is located on human chromosome 1q44 and consists of 9 coding exons [44]. NLRP3 inflammasome complex consists of NLRP3, ASC (apoptosis-associated speck-like protein containing a CARD) adaptor and caspase-1. NLRP3 contains amino terminal PYD to allow homotypic interactions with the PYD domain of ASC, a central NACHT domain, which is responsible for self-oligomerization, and LRR at the carboxy terminus, which modulates NLRP3 activity via its auto-inhibitory property and senses microbial ligands and endogenous alarmins [45]. The NLRP3 inflammasome is one of the most well studied inflammasome complexes in terms of function, signaling pathways, and role in disease pathogenesis.

Another feature of SLE is the production of autoantibodies against different autoantigens which were found in apoptotic and necrotic cells. Some of these autoantibodies show a linkage between...
Cell penetrating peptide derived from eosinophil cationic protein could downregulate HDM allergen-induced NLRP3 inflammasome activation

Cell penetrating peptide (CPP) has been used to treat allergic diseases in recent years [61, 62]. The first CPP to be identified was TAT peptide in 1989, corresponding to the basic domain of HIV-1 Tat protein [63]. Penetratin, corresponding to the third helix of the Antennapedia homeodomain, was identified in 1994 [64]. Since then, various peptides showing the same capacities have been identified or rationally designed. In this study, 10-residue peptide-CPPecp was derived from the human eosinophil cationic protein (ECP). ECP is a secretory ribonuclease (RNase) released by activated eosinophils and it has antiviral and antiparasitic activities [65]. In addition, ECP binds lipopolysaccharides and peptidoglycans tightly [66]. The N-terminal domain of ECP (residues 1–45) retains most of the antimicrobial properties [67]. CPPecp has been shown to internalize into bronchial epithelial cells [68]. In our previous study, THP-1 cells and PBMCs derived from HDM allergic patients were co-cultured with Der p 2 (1.5μg/ml) and Der p 2 (1.5μg/ml) for six hours. The results showed that the concentration of IL-1β was significantly downregulated in THP-1 cells and PBMCs (fig.5 and fig.6, respectively)[34]. The mechanism of inhibition of inflammasome activation is achieved through two pathways: 1) upregulation of IFN-α production, and 2) induction of autophagy [69, 70]. Type I IFN signaling can directly inhibit NLRP3 inflammasome activation in a STAT-1-dependent manner or induce IL-10 production which could activate STAT3 in an autocrine manner to reduce levels of pro-inflammatory cytokines [71]. Thus, we further analyzed level of IFN-α in the supernatant of CD14+ cells after stimulation. The results showed that levels of IFN-α were significantly upregulated in cells co-cultured with 50μM CPPecp and 1.5μg/ml of Der p 2(fig.7) [34].

Conclusion

Allergy and autoimmunity disease are traditionally considered as two distinct disease entities related to the development of either TH2-dominated or TH1-dominated immune responses. However, recent observations indicate that autoimmune disorders and allergies are more closely interconnected than has been hitherto thought. Both diseases can be initiated by environmental allergens and can activate immune responses, followed by unbalanced immunological tolerance in both TH1- and TH2-driven mechanisms. Furthermore, environmental factors such as HDM major allergen could induce autoantigen and autoantibody production and inflammasome activation through TLR and NLR in SLE patients. CPPecp was demonstrated to downregulate Der p 2-induced inflammasome activation via upregulating IFN-α expression. These findings suggest that environmental allergen might play a role in the pathogenesis of autoimmune disease and the modulation of inflammasome activation caused by allergen might be important in the prevention of autoimmune disease.

Acknowledgements

This study was based in part on data obtained from the National Health Insurance Research Database provided by the Bureau of National Health Insurance and managed by the National Health Research Institutes, Taiwan (Registration number NHIRD-99-315). The interpretation and conclusions contained herein do not represent those of the Bureau of National Health Insurance, or the National Health Research Institutes, Taiwan.


