A revisit to cockroach allergens

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Summary

Among cockroaches (CR) that live in people's homes, two species, *i.e.,* German CR (*Blattella germanica*) and American CR (*Periplaneta americana*) predominate in temperate and tropical areas, respectively. CR is an important source of inhalant indoor allergens that sensitize atopic subjects to (localized) type I hypersensitivity or atopy including allergic rhinitis and atopic asthma. In Thailand the predominant CR species is *P. americana*. CR allergens are found throughout CR infested houses; the number found in kitchens correlates with the degree of CR infestation while sensitization and reactivation of the allergic morbidity are likely to occur in the living room and bedroom. Levels of the CR allergens in homes of CR allergic Thais, measured by using locally made quantification test kits, revealed that the highest levels occur in dust samples collected from the wooden houses of urban slums and in the cool and dry season. CR allergens are proteins that may be derived from any anatomical part of the insect at any developmental stage. The allergens may be also from CR secretions, excretions, body washes or frass. The proteins may be the insect structural proteins, enzymes or hormones. They may exist as dimers/multimers and/or in different isoforms. Exposure to CR allergens in infancy leads to allergic morbidity later in life. Clinical symptoms of CR allergy are usually more severe and prolonged than those caused by other indoor allergens. The mechanisms of acute and chronic airway inflammation and airway hyper-responsiveness (AHR) have been addressed including specific IgE- and non-IgE-mediated mechanisms, *i.e.,* role of protease-activated receptor-2 (PAR2). Participation of various allergen activated-CD4$^+$ T cells of different sublineages, *i.e.,* Th2, Th17, Th22, Th9, Th25, Tregs/Th3 as well as invariant NKT cells, in asthma pathogenesis have been mentioned. The diagnosis of CR allergy and the allergy intervention by CR population control are also discussed. *(Asian Pac J Allergy Immunol 2010;28:95-106)*

Key words: *Cockroach,* Periplaneta americana, Blattella germanica*, cockroach allergy, cockroach allergens, Per a 1, ariginine kinase, proteases, atopy, allergic rhinitis, atopic asthma, airway remodeling, airway hyperresposiveness (AHR), IgE, $Fc_{\varepsilon}RI$, $Fc_{\varepsilon}RII$, Th2, Th17, Th9, Th25, IL-17, IL-9, protease activated receptors (PARs), cockroach avoidance, cockroach allergen detection, cockroach allergen quantification, G-protein couple receptor*

Abbreviations:

AHR = Airway hyperresponsiveness
APC = Antigen presenting cells
BHR = Bronchial hyperreactivity
CGRP = Calcitonin gene-related peptide
CR = Cockroach (-es)
DAG = Diacylglycerol
ECF = Eosinophil chemotactic factor
FGFs = Fibroblast growth factors
GST = Glutathione-S-transferase
HDM = House dust mites
iNKT = Invariant NKT
IP_3 = 1,4,5 inositolphosphate
ITAM = Immunoreceptor tyrosine activation motif
MAP = Mitogen-activated protein
MBP = Major basic protein
PAF = Platelet activating factor
PAR = Protease-activated receptor
PC = Phosphatidylcholine
PE = Phosphatidylethanolamine

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PLC = Phospholipase-C
PS = Phosphatidylserine
RAST = Radio-allergosorbent test
SNARES = Soluble N-ethylmaliemide adhesion receptors
SPT = Skin prick test
Tregs = Regulatory T cells
TRPV1 = Transient receptor potential vanilloid receptor-1
TSLP = Thymic stromal lymphopoietin

Introduction

About cockroaches

There are more than 3,500 cockroach (CR) species on earth. Fortunately, only a few species live in people's homes, including American CR (*Periplaneta americana*), German CR (*Blattella germanica*), oriental CR (*Blatta orientalis*), brown-banded CR (*Supella longipalpa*) and smoky brown CR (*P. fulliginosa*). Figure 1. shows adult German and American CR which are the predominant species in human dwellings. The German CR is light brown in color. The full sized-body length is ~16 mm with dark brown band on the protonotum. They are more common in cool, dry climates, *e.g.,* Europe and USA. The American CR prefers hot and humid conditions, such as those found in Brazil, Taiwan and Thailand. The adult American CR is much bigger than the German CR. The body length of the adult is ~38 mm with brownish-red color. They can fly only in short distance. In 2004, Tungtrongchitr *et al.* made an intensive survey of CR species in human dwellings in Bangkok and found, in falling order of percentage: 72.15, 2.75, 0.78, 0.78, 0.78, 0.39 and 22.3 of *P. americana*, *S. longipalpa*, *P. brunnei*, *P. australasiae, Neostylopyga rhombifolia, B. germanica* and nymphs of which the species could not be identified, respectively.[1] Usually cockroaches are fertile and thrive very well. Their life span is approximately 3-15 months. Most cockroaches that live indoors have a similar reproductive cycle. The female lays ~ 300 eggs. Eggs cases (ootheca) contain 10 to 50 eggs and stick to the CR environment, except those of the German CR which adheres to the female abdomen until 1-2 days before hatching. After hatching the nymphs undergo several molts before reaching the full maturity.

CR allergy

Allergic rhinitis and atopic asthma, which are due to immediate hypersensitivity of the upper and the lower respiratory tract, respectively, are the two most common human diseases attributable to CR infestation in the human environment. Exposure to CR early in infancy leads those with genetic propensity (atopic subjects) to become sensitized and to subsequently clinical manifestations upon re-exposure to the CR allergen.[2] There has been a report that as many as one-fourth of children aged less than 4 years old in Chicago, USA, were allergic to CR and 14% of them were allergic to CR alone.[3] The CR allergens may be species specific, common to all CR species, or common also to other insects such as bees and other invertebrates such as house dust mites (HDM) and shrimp (pan invertebrate allergens).[4] More than 45 years ago, it was noted that a skin rash appeared soon after the CR crawled over that part of skin. It was in 1964 that Bernton and Brown (1964) confirmed the existence of CR allergy. They found that 44% of 755 allergic patients who were treated at the allergy clinics in New York had positive by skin prick test (SPT) to CR extract and 13 % of those subjects were allergic to CR alone.[5] Bernton *et al.* performed a bronchial challenge test in 10 CR allergic patients using CR extract and found that all subjects developed immediate hypersensitivity.[6]

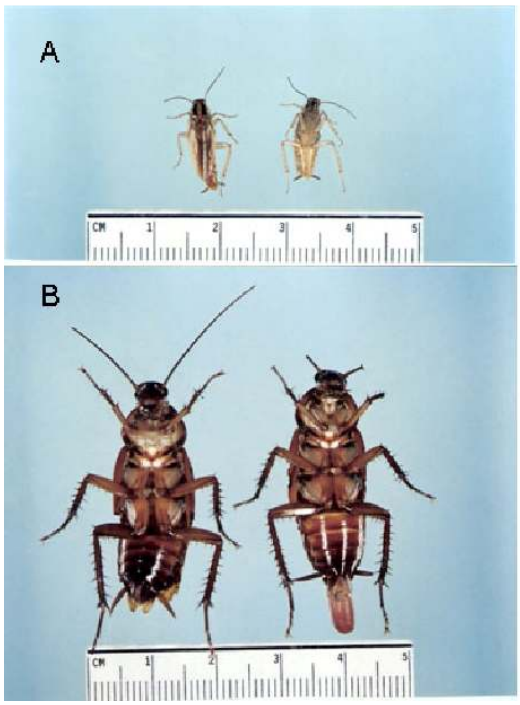

Figure 1. (A) Adult German and (B) American CR which are the predominant species in human dwellings (Courtesy Assoc. Prof. Dr. Anchalee Tungtrongchitr).

Table 1. Names, characteristics and functions of allergens from German and American cockroaches.

Allergens	Biochemical name	Molecular weight (KDa)	Allergenicity
Blattella germanica			
Bla g 1	-*	21-56	30-50 [a,b]
Bla g 2	Aspartic protease	36	60-80 [a,b]
Bla g 4	Calycin/lipocalin	21	30-77 [a,b]
Bla g 5	Glutathione S-transferase	22	70 [a,b]
Bla g 6	Troponin-C	18-21	14-46 [b]
Bla g 7	Tropomyosin	31	16 [b]
Periplaneta americana			
Per a 1	-*	13-45	80-100[a,b]
Per a 2	Aspartic protease	36	-*
Per a 3	Arylphorin(inactive) /Arthropod hemocyanin	72, 78	47-83[a,b]
Per a 4	Calycin/lipocalin	21	59[b]
Per a 6	Troponin-C	18	-*
Per a 7	Tropomyosin	33-37	43-57[b]
Per a 9	Arginine kinase	43	100[b]
Per a 10	Serine protease	28	80 [a,b]
Troponin-T	Troponin-T	47-50	17[b]

* No information

[a] Positive skin prick test

[b] Positive IgE binding reactivity

In 1979, Kang *et al.* demonstrated that CR allergic subjects developed asthma soon after inhaling CR extract.[7] These subjects also had high eosinophilia 24-48 hours later.[8] Thus asthma caused by CR is allergen specific similar to other types of atopic asthma.

Cockroach allergens and their biological activities

By definition, allergens are non-parasite proteins or chemicals that associated with proteins which induce massive IgE production in atopic individuals. These substances induce other immunoglobulin isotypes such as IgM and/or IgG and minimum, if any, IgE production in non-atopic subjects.[9]

CR allergens are antigenic proteins which may be glycosylated and exist in multimeric forms. They range in molecular sizes from 6 to >120 kDa. They are soluble within the host. CR allergens may be from any anatomical part and developmental stage of the insect, such as cuticle, dead body debris, eggs and egg cast. They can also be from various fluids such as hemolymph, regurgitating fluid, urine, feces, body washes and frass. Many allergens are insect enzymes or hormones. The CR allergenic particles (>10 microns) likely to be found on surfaces such as floors, lamps, tables, but are easily disturbed and disseminated by wind. Lists of allergens derived from German and American CR are shown in Table 1**.** The data are from studies of either native CR proteins or their recombinant counterparts produced from transformed bacteria or yeasts.

Data on allergenicity of individual proteins were gathered from the results of experiments performed either *in vivo* by using SPT or *in vitro* by determining the IgE binding activity of the proteins using radio-allergosorbent test (RAST), IgE-ELISA and/or IgE-immunoblotting. The amount of CR allergen specific IgE in the patient's serum can be quantified by using CAP-RAST.

The nomenclature of an allergen is written by using the first three letters of the name of the genus of the organism from which the allergen is derived followed, with a space in between, by the first letter of the name of the species and the designated number of the allergen from that species, respectively.

*Allergens from German CR (***Blattella germanica***)*

Bla g 1 is the first reported allergen derived from the German CR.[10,11] It is an isoallergen as there are several allergenic isoforms, *i.e.,* Bla g 1.0101[12], Bla g 1.0102 or Bla g bd90k[12-14], Bla g 1.0103[12], and Bla g 1.02.[12] The Bla g 1 isoallergens range in molecular sizes from 21-56 kDa. They have 70-72% amino acids which are identical to Per a 1 of the American CR. Both Bla g 1 and Per a 1 are regarded as group 1 cross-reactive CR allergens which also includes those of *Blatta orientalis, Supella longipalpa* and *P. fulliginosa*. The molecule of the group 1 CR allergens consists of tandem repeats of about 100 amino acids. Bla g 1 is a two duplex molecule. Each duplex consists of two polypeptides with 26-

29% amino acid homology.[12] Both Bla g 1 of German CR and Per a 1 of American CR have 30% amino acid homology to ANG12 protein of female *Anopheles gambiae,* which the female mosquito secretes after a blood meal indicating that the protein has role in nutrient acquisition, digestion and/or absorption of food.[12] Bla g 1 is also found in feces of both males and females but the amount is more in the female feces.[15] Bla g 1 elicits a positive SPT in 30-50% of CR allergic subjects[10,11] All of the duplex peptides could bind IgE in the CR allergic patients' sera implying that all peptides are allergenic.[12]

Bla g 2 is the aspartic protease of *B. germanica* and the molecular structure is similar to that of other aspartic proteases such as pepsin, chymosin and cathepsin of humans, *Aedes egypti* mosquitoes and *Drosophila melanogaster*.[4,16,17] Bla g 2 is a zinc metalloprotease with the molecular size ~36 kDa. The protein is abundant in the CR digestive tissues but can be found also in the feces and CR washings.[4] This allergen binds to IgE in 60-80% of CR allergic subjects[4]; thus Bla g 2 is a major allergen of the *B. germanica.* As low as 0.33 μg of the Bla g 2 per gram of dust, could sensitize an atopic individual for specific IgE production.[18]

Bla g 4 is the first CR allergen (similar to Per a 4 of the American CR) for which the encoding gene has been cloned and the three dimensional and crystal structures of the recombinant protein have been extensively studied.[19] Bla g 4 is a ligand binding protein similar in structure and activity to other calycins and lipocalins. Allergens similar to the Bla g 4 include mouse allergen, Mus m 1[20], α2-globulin of rat which is a protein for pheromone transportation[21], Can f 1 and Can f 2 from dog epithelium[22], Bos d 2 and Bos d 5 from ox[23,24], Equ c 1 from horse[25-28] and beta-lactalbumin from cow's milk. Lipocalins have several biological activities including nutrient acquisition, nitric oxide transport, insect colorization and development of the neuronal tissue of embryo.[29] Bla g 4 binds IgE in 40-60% of CR allergic subjects.[4]

Bla g 5 (22 kDa) is glutathione-S-transferase (GST). Currently, 3 isoforms of Bla g 5 have been described including GST1, GST2 and GST3. The Bla g 5 has 40-50% amino acid homology to GST of other insects and 28% homology to Der p 8 of HDM, *Dermatophagoides pteronyssinus*.[30] GST is an insect enzyme used for detoxification of both endogenous and exogenous toxic compound, *e.g.,* insecticide. About 70% of sera of German CR allergic subjects contained IgE that bound to the protein.[30]

Gene coding for Bla g 6 or troponin-C (a calcium binding protein which functions in muscle contraction) of German CR was cloned and the recombinant protein, 18-21 kDa, was produced. The recombinant troponin-C bound IgE in 14-46% of CR allergic subjects.[31,32]

Bla g 7 or tropomyosin (31 kDa) is also a calcium binding protein. Tropomyosin is regarded as a pan-invertebrate allergen, as Bla g 7 showed more than 90% amino acid identity with Per a 7 of American CR and shrimp tropomyosin.[33-35] However, Bla g 7 bound IgE in only 16% of CR allergic patients[35]; thus the protein is a minor German CR allergen.

Allergens from American CR (*Periplaneta americana*)

Per a 1 is an isoallergen with 5 isoforms reported so far, *i.e.,* Per a 1.0101-Per a 1.0105.[14,36-38] Gene coding for Per a 1 was cloned, sequenced and the recombinant protein was produced from transformed bacteria and yeasts.[14,37-39] Recombinant Per a 1 (13.8 kDa) tends to form dimers.[38] Per a 1 is a major American CR allergen as it bound to IgE in the sera of 90-100% CR allergic subjects.[36,38] In 2003, Diraphat *et al.* cloned a full length sequence of gene coding for *P. americana* caught in Thailand (Per a 1.0105; accession no. AY259541) and demonstrated a 372 open reading frame which were deduced into a 124 amino acid, 13.8 kDa and pI 4.74. All sera of the CR allergic Thais who were positive by SPT to CR extract contained IgE that bound to the recombinant Per a 1.0105. The Per a 1.0105 has an allergenic epitope (LIRSLGLP) that differs in only one amino acid from the previously reported two epitopes (LIRALFGLP and LIRSWFGLP) of the Per a 1.0104 which bound to IgE in sera of 80 and 100% of CR allergic subjects, respectively. The Per a 1.0105 contains both hydrophilic and hydrophobic portions indicating that it might be a transmembrane protein.[38]

Per a 2 is an inactive aspartic protease[40] with about 44% amino acid sequence identity to Bla g 2. The protein is found in the American CR digestive tract and feces.

Per a 3 (72 kDa) is a CR storage protein. Currently, 4 isoforms of this isoallergens have

been reported including Per a 3.01, Per a 3.0201, Per a 3.0202 and Per a 3.0203. The protein has 27-36% sequence identity to arylphorins, hemocyanin and insect embryonic hormone.[41,42] Per a 3 extracted from American CR (native protein) elicited skin reactivity in 83% of CR allergic patients while only 47% of the patients were positive by SPT to recombinant Per a 3.[37] The Per a 3.01 allergenicity and cross-reactivity with the Bla g 3 have been investigated.[36,43]

Per a 4 is a lipocalin protein similar to Bla g 4 and functions in pheromone secretion in male American CR.[19] About 60% of patients with allergic rhinitis and asthma in Singapore have been sensitized by Per a 4 indicating that Per a 4 is another major American CR allergen.[44]

Per a 6 or troponin-C of the American CR is an 18 kDa protein for which the data on allergenicity is as yet unavailable.

In 2007, Khantisitthiporn *et al.* cloned full length gene sequence coding for troponin-T of an American CR caught in Thailand and prepared the recombinant as well as native troponin-T proteins. It was found that the native troponin-T bound IgE in sera of 17% of CR allergic Thais while the recombinant protein did not have any detectable IgE binding activity implying that the native troponin-T of American CR is a minor CR allergen. [45]

Per a 7 or tropomyosin of American CR (33 kDa) has a high sequence identity to tropomyosins of other invertebrates, including shrimp (82%), mollusk and Der p 10 and Der f 10 of *D. pteronyssinus* and *D. farinae*, respectively, (80%)[46,47]; thus, tropomyosins are pan invertebrate allergens. Recently, Sookrung *et al.* cloned and sequenced the full length gene coding for *P. americana* tropomyosin (accession no. FJ976895).[48] The native and recombinant Per a 7 bound IgE in the sera of 57% and 43% of CR allergic Thais, respectively. Thus, native Per a 7 is another major American CR allergen.

Per a 9, or arginine kinase, of *P. americana* (43 kDa) is another major American CR allergen as the protein purified from American CR extract by monoclonal antibody based-affinity chromatography reacted with IgE in sera of all CR allergic Thai patients.[49]

Per a 10, or serine protease (28 kDa), gave skin reactivity in 80% of CR allergic subjects.[50]

Clinical manifestations of cockroach allergy

CR is the cause of localized IgE-mediated (type I) hypersensitivity or atopy. The most common forms are allergic rhinitis and asthma. The CR allergy may affect both children and adults and the world incidence ranges from 40-75%.[4-7,51-53] Predisposing factors to the allergy are genetic and environmental. Inhabitants of crowded inner city areas, belonging to low socioeconomic groups are at the higher risk to CR sensitization.[54] Symptoms of CR allergy may range from urticaria, atopic dermatitis, hay fever, rhinitis, nasal congestion, rhinorrhea, lacrimation, sneezing, coughing, to chest tightness, breathlessness and asthma which may require hospitalization or emergency room visits. Most CR sensitized subjects have serum IgE that bind to CR allergens.

Mechanisms of allergic rhinitis and asthma

CR is a major source of inhalant indoor allergen. CR allergens are found throughout the house including the kitchen, dining room, living room and bedroom. However, most people are not aware of the presence of the allergens in their environment and pay no or minimum attention to the initial symptoms of CR allergy. Prolonged exposure and sensitization to CR often lead to severe allergic rhinitis and asthma. The threshold level of CR allergen for sensitization has been estimated at 2 U per gram of dust[55] while the morbidity threshold is likely to be 8 U per gram of dust.[2]

The underlying mechanisms leading to CR allergic morbidity include:

Type I hypersensitivity[9] or atopy: respiratory mucosa is the first human tissue that contacts with the inhaled allergens. Both myeloid and plasmacytoid dendritic cells (DCs) are abundant in the respiratory mucosa. They have been exposed to the mucosal environment, such as thymic stromal lymphopoietin (TSLP) produced by the respiratory epithelial cells, and have a propensity to engage in either muscosal tolerance or Th2 response. The DCs endocytose the allergenic protein which travels to nearby lymphoid tissue. On the way they express B7 (CD80 and CD86) and MHC class II molecules and loose their phagocytic activity; thus they become antigen presenting cells (APC). After being processed and assembled into the groove of an MHC class II molecule, the allergenic peptide is presented to $CD4^+$ T cell and under appropriate

co-stimulation in a cytokine milieu the T cell proliferates and differentiates into allergen specific Th2 cells which produce typical Th2 cytokines, including IL-4, IL-5, IL-13, IL-21 and IL-31. IL-4 and IL-13 influence class switching of the allergen specific B cells to produce IgE. The IgE then becomes fixed to IgE receptors on the surface of mast cells which are abundant in the respiratory mucosa (or basophils derived from the blood circulation) *via* both CH3 domains of the Fc portion of IgE molecule. There are two types of human IgE receptors. Type I receptors or $Fc_{\varepsilon}RI$ bind with high affinity to the IgE Fc portion. This membrane receptor consists of four trans-membrane polypeptides including one each of α and β chains and two identical γ chains. Each α chain has extracellular, trans-membrane and cytosolic portions. The extracellular portion consists of two immunoglobulin-like domains which bind the CH3 domains of IgE. The β chain is a polypeptide that traverses the membrane four times and lies between the α and the γ chains. The cytoplasmic portion of the β chain is associated with a Src family kinase called Lyn, which functions similarly to Lck in the T cell. The two identical γ chains are equivalent to the ξ chains of T cell or Igα/Igβ of the B cell. The extracellular portions are short and linked together by a disulfide bond. The cytoplasmic portions of the γ chains are long and contain one each of the immunoreceptor tyrosine activation motif (ITAM).[56] $Fc_{\varepsilon}RII$ or CD23 binds to IgE Fc portion with relatively lower affinity than the $Fc_{\varepsilon}RI$. $Fc_{\varepsilon}RII$ is a trans-membrane polypeptide. The extracellular portion acquires a domain of C-type lectin which is different from the extracellular domains of the $Fc_{\varepsilon}RI$. There are two $Fc_{\varepsilon}RII$ isoforms; CD23a is found on B cells and another isoform is found on the surface of other cell types after the cells are stimulated by IL-4. Cross-linking of surface CD23 by allergen leads to stimulation of the respective cells including B cells, macrophages and eosinophils.[9] Soluble CD23 (sCD23) derived from enzymatic digestion of the extracellular portion of membranous $Fc_{\varepsilon}RII$ is found in serum and the level is higher in atopic subjects than in non-atopic counterparts.[9]

Cross-linking of adjacent IgE molecules that are fixed to the Fc_{ε} receptors leads to mast cell degranulation. Immediately after the IgE cross-linking, Lyn kinase at the cytoplasmic tails of β chains of adjacent (clustered) Fc_{ε} receptors becomes active and phosphorylates several protein tyrosine kinases, such as Fyn, Syk, Lyn, which give rise to several secondary messengers inside the cell. Several phospholipases, such as PLC and PLA_2, located at the plasma membrane become active also. The PLC converts PIP_2 to yield diacylglycerol (DAG) and 1,4,5 inositolphosphate (IP_3). The DAG activates inactive protein kinase C while IP_3 releases intracellular Ca^{++} from the endoplasmic reticulum depot into the cytoplasm. Microtubules between mast cell granules and the plasma membrane are formed as a result of DAG and Ca^{++} activity. Membrane phosphatidylserine (PS) is converted to phosphatidylethanolamine (PE). Methylation of PE by phospholipid methyltransferases yields phosphatidylcholine (PC) in the membrane which causes an increase in membrane fluidity that facilitates the granule release. There was also a transient rise of intracellular cAMP which activates cAMP dependent protein kinase causing swelling of the mast cell granules with increased membrane fluidity. The subsequent drop of the cAMP level allows completion of the degranulation process. Various pre-formed mediators are then released into the extracellular milieu by a mechanism similar to the release of neurotransmitter, *i.e.,* acetylcholine, of the motor-nerve junction which occurs *via* the SNARE fusion complexes formed by the soluble N-ethylmaliemide adhesion receptors (SNARES) on the granule and the plasma membranes. Mitogen activated protein (MAP) kinase together with Ca^{++} stimulates PLA_2 to convert PC to lyso-PC and arachidonic acid. The latter is metabolized to yield various leukotrienes, prostaglandins and platelet activating factor (PAF). Moreover, downstream signals of the activated MAP kinase give rise to expression of various cytokine genes and production and secretion of various mast cell cytokines including TNFα, GM-CSF, IL-1β, IL-3, IL-4, IL-5 and IL-13.[9]

Various symptoms of type I hypersensitivity occur as a result of biological activities of the mediators released from mast cell (also basophil). These mediators affect local tissues as well as various other secondary cells including eosinophils, neutrophils, T cells, monocytes and platelets. The mast cell mediators are classified into primary and secondary mediators. Primary mediators are pre-formed and stored in the mast cell cytoplasmic granules. These include

histamine, various proteases, eosinophil chemotactic factor (ECF), neutrophil chemotactic factor (IL-8) and heparin. These mediators have different biological activities: histamine and heparin increase vascular permeability causing tissue edema and smooth muscle contraction. ECF and IL-8 attract the respective cells into the respiratory tissue. Proteases such as tryptase and chymase increase mucus secretion and stimulate many receptors on various cells of the respiratory tissue resulting in inflammation and damage of the tissue basement membrane and blood vessels. Secondary mediators include phospholipid metabolites (leukotrienes, prostaglandins, PAF) and products of the infiltrated secondary cells (eosinophils, neutrophils, T cells, monocytes, and platelets), *i.e.* cytokines, chemokines, enzymes and bradykinin. IL-1 and TNFα increase cell adhesion molecules (CAMs) on vascular endothelium resulting in an increase in white blood cell extravasation and respiratory tissue infiltration. PAF causes platelet aggregation and degranulation resulting in smooth muscle contraction especially in lower respiratory tissue and lungs. Leukotrienes, especially LTC_4, increase vascular permeability causing edema and smooth muscle contraction. Prostaglandins, *e.g.,* PGD_2, cause vascular dilatation, increase vascular permeability, smooth muscle contraction and platelet aggregation. IL-4 and IL-13 stimulate B cells to produce more IgE while IL-5 increases eosinophil generation, differentiation and survival.[9]

Allergic rhinitis is an ultimate effect due to biological activities of various mast cell mediators which act upon the mucosa of upper respiratory tract, *i.e.,* nasal cavity and sinuses, as well as the conjunctivae. Blood vessels of these mucosae are congested, dilated and the vascular permeability is increase. Symptoms of the allergic rhinitis include nasal blockade, rhinorrhea, lacrimation, pruritus and frequent coughing and sneezing.

Atopic asthma is a result of inflammation of the lower respiratory mucosa and lungs. The clinical manifestations include wheezing, chest tightness and shortage of breath because of the bronchial muscle contraction, airway tissue edema and excessive mucus in the respiratory tract. The asthmatic's responses to the allergen may be divided into "early" and "late" responses. The early response occurs soon (within minutes) after the allergen sensitized patient inhales the allergen and are mainly due to the mast cell pre-formed mediators and lipid metabolites such as histamine, heparin, leukotrienes and prostaglandins. The late response occurs many hours later and usually lasts a day or two or sometimes longer. Additional mediators including various cytokines and chemokines play an important role in the late response of asthma. Endothelial cells of the inflamed respiratory tissues express more cell adhesion molecules facilitating leukocyte adhesion, extravasation and infiltration into the airway. The principal inflammatory cells of the late response are eosinophils and neutrophils. These cells cause tissue damage by releasing toxic enzymes such as proteases, neutrophil elastase and myeloperoxidase, eosinophil neurotoxin, eosinophil major basic protein (MBP), chemokines, cytokines and oxygen radicals, which cause local tissue damage and mucin metaplasia (columnar cells with excessive cytoplasmic mucin). The airway mucus plugs contain mainly the damaged and sloughed-off epithelial cells, mucus proteins, inflammatory cells and bronchial spirals called Curschmann's spirals.[9] Chronic inflammation of the respiratory tissue often causes tissue remodeling leading to a state of airway hyper-responsiveness (AHR) or bronchial hyper-reactivity (BHR).

The airway hyper-responsiveness (AHR) or bronchial hyper-reactivity (BHR) is a sequel of prolonged asthma symptoms and chronic respiratory tissue inflammation. The respiratory tissues are damaged and the overall process of reparation leads to the tissue remodeling characterized histologically by thickening of the bronchial basement membrane due to peribronchial fibrosis and narrowing of the respiratory canal which contains thick mucus plugs. The lung function is reduced and the airway tissue is highly sensitive, not only to the allergen but also to other inhaled substances. The airway remodeling is believed to be due, on one hand, to the damaged/inflamed airway structural tissue, including epithelium (epithelial cell sloughing-off, chemokine production and mucin metaplasia), subepithelial smooth muscles (hyperplasia) and fibroblast (excessive collagen production causing fibrosis), and, on the other hand, to the infiltrating immune cells including various T cell subsets (please see below), B cells, macrophages, eosinophils, neutrophils and mast cells.

For many decades, antigen stimulated naïve $CD4^+$ T cells have been known to differentiate into two functionally different subsets of T-helper cells, *i.e.,* Th1 and Th2, depending upon the signals generated through the TCR and the cytokine milieu.[57,58] IL-12 from innate immune cells, such as macrophages, signals the naïve $CD4^+$ T cell through STAT4 to differentiate to IFNγ producing Th1 cells. IL-12 and the IFNγ, through STAT4 and STAT1, drive the Th1 cells to express T-bet transcription factor which enhances more IFNγ production and also the secretion of other cytokines, *i.e.,* IL-2, TNFβ and IL-10. Th1 cytokines play important role in cellular immunity and promote IgG2a class switching. Th1 cells are also known to be involved in certain autoimmune inflammations.[59] Th2 cells are derived from the naïve $CD4^+$ T cells under the influence of IL-4 secreted mainly by tissue mast cells. The IL-4 which signals through STAT6 drives the expression of GATA3 transcription factor in the naïve T cells and commits them to Th2 lineage. The principal cytokines secreted from the Th2 cells include IL-2, IL-4, IL-5, IL-13, IL-21 and IL-31 which mediate immunity to parasitic infections as well as immunopathogenicity of the allergic diseases such as allergic rhinitis and asthma by inducing IgE production and eosinophil generation and maturation. During the past two decades, however, other T helper cells that are involved in immune regulation, various inflammatory diseases (including autoimmune disorders and allergy especially severe asthma pathogenesis) and respiratory tissue remodeling, have been described. These include regulatory T cells (Tregs), Th17, Th22, Th9 and Th25. Moreover, invariant NKT (iNKT) cells have also been implicated in the development of allergic disease as they also secrete IL-4, IL-13, IFNγ and TNF as well as IL-17, IL-22 and IL-9 upon being stimulated *via* the TCR.[60-62] Detail information on these cells has been reviewed extensively elsewhere.[62] Their roles in asthma pathogenesis are given briefly below.

The human Th17 cell is another type of T-helper cells. They develop from naïve $CD4^+$ T cells under the influence of IL-23 which signals the naïve T cells through STAT3 rendering the cell to express retinoic acid orphan receptor or $ROR_{a/c}$ transcription factor (RORγt in mice).[62,63] These Th17 cells typically produce IL-17, IL-17A, IL-17F, IL-22, IL-26, TNFα, CCL20 as well as lymphotoxin-β.[64] Much progress has been made towards understanding the role of Th17 cells in airway diseases and asthma pathogenesis.[63,65-68] IL-17 mRNA and the expressed protein (IL-17A) have been found to increase in the airway tissues of asthmatic subjects.[65,69] IL-17 induces the release of cytokine, chemokine and growth factors including IL-6, IL-8, C-X-C chemokine, GM-CSF and growth-related oncogene-α (GRO-α) from epithelial and smooth muscle cells of human airway tissue.[70-74] Th17 cytokines, IL-17A and IL-17F, recruit neutrophils into the airway, prolong their survival and produce pulmonary neutrophilia which amplifies the allergen-induced allergic response.[75] IL-17 also increases elastase and myeloperoxidase activity of the neutrophils,[76] induces fibroblasts to produce cytokines[65] and increases matrix metalloprotease-9 in the allergic airway.[77] The above mentioned biological activities of Th17 cell products indicate that Th17 cells have important role in causing severe asthma and airway remodeling which lead to AHR[62,69,78]

Th22 is another subset of human T helper cells which has been identified only recently.[79] Th22 cells differentiate from $CD4^+$ T cells in the presence of TNFα and IL-6. They are believed to be a terminally differentiated and distinct T cell subtype.[79] Th22 cells express CD4 and TCR but not NK cell marker; thus they are non-NK cells. Th22 cell clones secrete predominantly IL-22, fibroblast growth factors (FGFs) and moderate amounts of TNFα and IL-10; but do not secrete IFNγ, IL-4 or IL-17.[79] Th22 cells have been found to be involved in the innate immunity of skin (antimicrobial)[80] and wound healing and remodeling of epithelial barrier.[79,81,82] Because elevated levels of the IL-22 are found in asthmatic patients and because cells other than Th22, including Th17[68] and a subset of NK cell (NK22)[83], also secrete IL-22, the role of Th22 in (?constraining) the asthma severity and airway tissue remodeling await detailed investigation.

Th9 cells are a new subset of activated $CD4^+$ T cells which dedifferentiated from Th2 under the influence of TGFβ and IL-4. Th9 cells are so-named because they produce large amounts of IL-9.[84] The role of Th9 and IL-9 in allergic diseases, especially severe asthma and airway remodeling, have been reviewed extensively elsewhere.[62,85]

Th25 is a new subpopulation of T helper cells that secrete IL-25.[62,86] IL-25, in similar way to IL-33,[62,87,88] stimulates other cells, *e.g.,* $CD11c^+$ DCs, T cells and iNKT cells, mast cells and basophils to produce large amounts of typical Th2 cytokines, *i.e.,* IL-4, IL-5, IL-13, which enhance allergen-induced airway inflammation[89] and prolonged eosinophil survival.[62,87,88]

Human invariant NKT cells ($CD161^+$, invariant TCRα chain NKT; iNKT) may be divided into two CD4 sublineages ($CD4^+$ and $CD4^-$) which produce different cytokine profiles. The $CD4^+$, $NK1.1^+$ NKT produce Th1 and Th2 cytokines, *i.e.,* IFNγ, IL-10, TNF, IL-4 and IL-13 where as $CD4^-$, $NK1.1^-$ NKT produce Th1 cytokines, *i.e.,* IFNγ and TNF.[60,90-92] NKT also produces high level of IL-17[93,94], IL-22 and IL-9.[61,95,96] Their role in IgE production, stimulation of pulmonary mast cell progenitor, airway neutrophilia, bronchial asthma and allergen-induced AHR has been observed.[90,93,96-98]

Regulatory T cells (Tregs) are another sublineage of $CD4^+$ T cells. Phenotypically, they have the CD25 signature which is the IL-2Rα chain ($CD4^+CD25^+$ cells) and express FoxP3 transcription factor. These cells require IL-2 for survival (another $CD4^+$ sublineage, *i.e.,* Th3 cells are similar to Treg but require TGFβ for differentiation). Tregs secrete TGFβ and IL-10 and function in maintaining self-tolerance. The big picture and the role of Tregs in suppression and treatment of allergic diseases including allergic rhinitis, asthma and AHR by means of Th2/Th2 cytokine manipulation have been investigated and reviewed elsewhere.[99-102]

Non-IgE-mediated airway inflammation

Besides the IgE-mediated inflammation of the airway tissue described above, proteases of the inhaled allergens, such as German CR aspartic protease (Bla g 2) and American CR serine protease (Per a 10), as well as several host endogenous proteases in the inflamed tissue, *i.e.*, mast cell tryptase and chymase, neutrophil cathepsin-G, tissue trypsins as well as coagulation factors, can signal the host cells through protease-activated receptors (PARs) leading to inflammation, tissue repair and pain.[103,104] PARs are G-protein couple receptors which are expressed on the membrane of a variety of human cells (as well as other mammalian). Currently, 4 different PARs have been described, *i.e.,* PAR1-PAR4. Nevertheless, all PARS are activated by proteases by the same mechanism. Proteases cleave a specific site located in the extracellular N-terminal portion of the PAR and exposed a tethered ligand that binds to a conserved region in the loop II of the trans-membrane portion.[105] Subsequent cellular events that follow depend upon the type of PAR and the cell that is activated. PAR2 are found in various human tissues and are abundant on epithelial and endothelial cells, immune cells, nerve cells, myocytes and fibroblasts of the respiratory tissue.[103,106-109] Signal transduction *via* PAR2 mediates phospholipase-C (PLC_β) activation with subsequent formation of DAG and IP3 similar to the surface IgE cross-link on the mast cells described above. Activated MAP kinase *via* PAR2 set a downstream signaling cascade inside the activated cells leading to several gene transcription and expression.[103,104,110] PAR2 activation results in a release of various inflammatory mediators including prostaglandins from lung epithelial cells.[104] PAR2 activation of Vagal pulmonary sensory nerve ending leads to activation of transient receptor potential vanilloid receptor-1 (TRPV1), which is a thermal and chemical transducer causing neurologic inflammation and hyperalgesia.[111] PAR2 activation releases calcitonin gene-related peptide (CGRP), substance P (SP) and neurokinin-A (NKA) causing blood vessel dilatation. PAR2 activation plays important role in airway inflammation and AHR.[112]

Diagnosis of CR allergy

Current diagnosis of CR allergy is made clinically based on the patient's circumstances (inhabitants of inner city, low socio-economic status, substandard housing, etc), clinical symptoms, and the result of skin prick test using crude CR extract. Measurement of CR allergen specific IgE in serum sample by immunological assays including CAP-RAST, IgE-ELISA and/or IgE-immunoblotting gives a presumptive diagnosis for CR allergy.

Intervention for CR allergy

The best preventive measure to avoid sensitization by CR allergens among naïve subjects and intervention of CR allergic morbidity among CR sensitized individuals is "CR allergen avoidance". Elimination of cockroach infestation/control of CR population in housing can be performed by physical and chemical

measures. Deprivation of the CR food and water can reduce/eliminate CR infestation. Human food should be kept in a refrigerator or in tight-lidded containers, snacks should not be left outside or in opened container, garbage must be kept in a closed container, pet food must not be left outside or open, kitchen and dining areas must be thoroughly cleaned to eliminate all food debris. Increased ventilation and elimination of moisture inside the house should be done on a regular basis. CR hiding places must be eliminated, *e.g.,* empty paper boxes, magazines, newspapers, etc. Tap water must not be left dripping; also there should not be water condensation on air conditioner pipe or leaking tap water pipe. The bottom of the sink must be closed when the sink is not in use. The house should be regularly and thoroughly cleaned. Several insecticides are available to kill CR but usually they cannot kill the CR eggs. Therefore insecticide spray should be repeated in order to eliminate the newly hatched nymphs. After intensive CR population control, the level of CR allergens should be satisfactorily reduced within 6 months. Quantification of CR allergens in dust collected from houses can be performed by using CR allergen quantification test kits.[1,113-115]

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