Allergens of *Blomia tropicalis*: An Overview of Recombinant Molecules

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Abstract

Allergic diseases are considered a major problem for health-care systems in both developed and developing countries. House dust mites are well-known triggers of allergic manifestations. While the *Dermatophagoides* genus is widely distributed globally, *Blomia tropicalis* is the most prominent mite species in the tropical and subtropical regions of the world. Over the last decades, an increase in sensitization rates to *B. tropicalis* has been reported, leading to increased research efforts on *Blomia* allergens. In fact, 8 new allergens have been identified and characterized to different degrees. Here, we provide an overview of recent developments concerning the identification and production of recombinant *Blomia* allergens, as well as their structural and immunological characterization. Although considerable progress has been achieved, detailed molecule-based studies are still needed to better define the clinical relevance of *Blomia* al-

lergens. Thus, the establishment of a well-standardized and fully characterized panel of allergens remains a challenge for the development of better diagnosis and therapy of allergic diseases induced by *B. tropicalis*.

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Introduction

The mite species *Blomia tropicalis* (Acari: Astigmata: Echimyopodidae) was originally identified in the 1970s and characterized as a storage mite by van Bronswijk and de Cock [1]. Moreover, this globule-shaped mite was especially found in storage facilities for grains and as contamination of processed food made from grains [1]. Nowadays it is well accepted that *B. tropicalis* also constitutes a very important house dust mite species, especially, but not exclusively, in the tropical and subtropical regions of the world [2–5]. In addition to allergen polymorphisms, some authors have claimed seasonal dynamic patterns of mite species, allergen densities within them, and different specific breeding sites [6–10]. All of these factors influencing the allergen prevalence could in turn have a direct

Table 1. Molecular properties of the *Blomia tropicalis* allergens

Allergen	MW ¹ , kDa	Protein function/family	PDB No.	Allergenicity test	IgE reac- tivity, %	Recombinant production/ organism	References
Blo t 1	25	Cysteine protease	–	SPT	2–93	<i>E. coli</i> and <i>P. pastoris</i>	11, 28, 30
Blo t 2	13	Unknown	–	ELISA	18–57	<i>E. coli</i>	11, 34
Blo t 3	24	Trypsin	–	SPT/ELISA	9–57	<i>E. coli</i>	11, 35
Blo t 4	55	α-Amylase	–	SPT	1–63	<i>P. pastoris</i>	11, 29, 40, 41
Blo t 5	14	Unknown	2JMH 2JRK 2MEY	SPT	12–98	<i>E. coli</i> , <i>P. pastoris</i> , and Chinese hamster ovary cells	21–23, 40, 41
Blo t 6	26	Chymotrypsin	–	SPT	0–77	<i>E. coli</i> and <i>P. pastoris</i>	40, 41, 46
Blo t 8	28	Glutathione S-transferase	4Q5N	SPT	10–100	<i>E. coli</i>	11, 58, 59
Blo t 10	33	Tropomyosin	–	SPT/ELISA	10–38	<i>E. coli</i>	11, 55, 61
Blo t 11	102	Paramyosin	–	Dot blot	5–87	<i>E. coli</i> and Sf9 insect cells	25, 40, 65
Blo t 12	14	Unknown	2MFK	RAST	0–70	<i>E. coli</i> and <i>P. pastoris</i>	6, 40, 41, 68
Blo t 13	15	Fatty-acid-binding protein	–	RAST	7–11	<i>E. coli</i> and <i>P. pastoris</i>	11, 27, 72, 74
Blo t 19	7	Antimicrobial peptide homolog	2MFJ	–	10	–	75
Blo t 21	11	Unknown	2LM9	SPT/ELISA	57–93	<i>E. coli</i>	11, 76

MW, molecular weight; *E. coli*, *Escherichia coli*; *P. pastoris*, *Pichia pastoris*. ¹ Calculated using ProtParam [114]. Only the sequence of the mature chain was used for the calculations. The sequences used for the ProtParam analysis were selected according to the following criteria: (i) sequence published in PDB database (<http://www.rcsb.org/pdb>), and (ii) in case the first criteria was not fulfilled, the isoform 0.0101 sequence published in the International Union of Immunological Societies database was used.

effect on the allergenicity of mites within different environments and/or periods of the year. Of note, an increase in *B. tropicalis* allergy has been observed over the last decades, changing immunoglobulin E (IgE) reactivities worldwide [3, 6, 11–13].

Moreover, in the tropical and subtropical areas of the world, the importance of *B. tropicalis* as a cause of sensitization has been demonstrated [2, 14–17] and even early life IgE responses to *B. tropicalis* have been reported [13, 18]. Therefore, the advances in knowledge regarding the allergenicity of *B. tropicalis* and its profile of reactive allergens are imperative for the design of clinical interventions as well as the diagnosis of *Blomia* allergy.

Up to now, out of 25 identified IgE-binding proteins of *B. tropicalis*, 13 have been officially acknowledged and named by the International Union of Immunological Societies (IUIS) nomenclature subcommittee [19]. These proteins are registered in the allergen database (www.allergen.org) and summarized in Table 1. These developments were enabled by the application of recombinant DNA technology, which opened up new possibilities for the field of allergy, leading not only to the production of purified recombinant house dust mite allergens but also to the standardization of molecule-based preparations for diagnosis and allergen-specific immunotherapy [20].

Since the first data of a purified recombinant *B. tropicalis* allergen (rBlo t 5) published in 1995 by Arruda et al. [21], several research groups have produced *B. tropicalis* allergens in recombinant form using different expression systems [12, 22–25].

Despite substantial advances in the last decades, knowledge about the biochemical and immunological properties of these 13 IUIS-acknowledged *Blomia* allergens has been derived from predictions based on data published for other homologous and well-characterized mite allergens [26–29]. Here, we review the current status of the production of recombinant *B. tropicalis* allergens, as well as of their structural and immunological characterization, with a special focus on: (i) secondary and tertiary structures, (ii) B- and T-cell epitopes, and (iii) cross-reactivity patterns.

Allergens of *B. tropicalis*

Blo t 1

The apparent molecular weight (MW) of the native Blo t 1 allergen in gel electrophoresis is 26 kDa and, so far, 2 isoforms have been produced as recombinant proteins [11, 28, 30–32]. Similarly to other group 1 mite allergens

Fig. 1. Tertiary structures of *Blomia tropicalis* allergens. Structures for Blo t 5, 8, 12, 19, and 21 were taken from the PDB database [111]. For the remaining allergens, models were calculated by comparative modeling using MODELLER software [51]. In case of nuclear magnetic resonance PDB entries, the model scoring function of MAESTRO was employed [112] to select a representative template structure. With MODELLER, 5 model variants were generated. The displayed structures always represent the model with the best MAESTRO score. Molecular graphics were created with UCSF Chimera [113]. Unless denoted otherwise, active site residues are shown in red and T-cell epitopes in purple. B-cell epitopes are represented as orange surface patches. In the case of comparative modeling, the UniProt code of the target allergen sequence, the organism of the template structure, and its PDB code are given in parentheses, otherwise only the PDB code is shown. **a** Blo t 1 (A1KXI0 modeled on 1XKG from *Dermatophagoides pteronyssinus*). The orange ribbon represents the pro-peptide, which blocks the access to the CYS-HIS-ASN catalytic site **b** Blo t 2 (Q1M2P1

modeled on 1KTJ from *D. pteronyssinus*). **c** Blo t 3 (Q8I916 modeled on 1GQD from *Fusarium oxysporum*) is a potential serine type endopeptidase with a SER-HIS-ASP catalytic triad. **d** Blo t 4 (A1KXI2 modeled on 1PIF from *Sus scrofa*) is a potential α -amylase, with ASP and GLU at the active site. **e** Blo t 5 (2MEY), T-cell and B-cell epitopes as described in other studies [42, 44, 47] are depicted. **f** Blo t 6 (A1KXI3 modeled on 2F91 from *Astacus leptodactylus*) is also a potential serine type endopeptidase. **g** Blo t 8 (4Q5N), chain A (in green), and chain B (in orange). **h** Blo t 10 and Blo t 11 are expected to contain extensive coiled coil regions. Here we show a coiled coil region from tropomyosin (3U59) from *Gallus gallus*. How the coils fold up in space cannot be reliably modeled on the currently known PDB entries. **i** Chitin-binding domain of Blo t 12 (2MFK). **j** Blo t 13 (Q17284 modeled on 2A0A from *D. farinae*) is a fatty-acid-binding protein. **k** Blo t 19 (2MFJ). **l** Blo t 21 (2LM9); the depicted residues have been shown to be a part of B-cell epitopes [79].

Table 2. Sequence alignment of *Blomia tropicalis* and *Dermatophagoides pteronyssinus* allergens

Allergens	Identity score, %
Blo t 1/Der p 1	35.6
Blo t 2/Der p 2	38.9
Blo t 3/Der p 3	54.8
Blo t 4/Der p 4	69.4
Blo t 5/Der p 5	44.3
Blo t 6/Der p 6	65.9
Blo t 8/Der p 8	35.5
Blo t 10/Der p 10	94.4
Blo t 11/Der p 11	89.3
Blo t 13/Der p 13	83.1
Blo t 21/Der p 21	37.2

The sequence alignment of each allergen group was determined using the CLUSTAL O (1.2.2) multiple sequence alignments program from the European Bioinformatics Institute (EBI) of the European Molecular Biology Laboratory (EMBL) (<http://www.ebi.ac.uk/Tools/msa/clustalo/>). The sequence of isoform 0.0101 of each allergen group was retrieved from the International Union of Immunological Societies database. Only the sequence of the mature chain of each allergen was used for the alignments.

[28, 30], Blo t 1 has also been classified as a cysteine protease. The crystal structure of a recombinant proBlo t 1 was recently determined [33]. Our molecular modeling, based on this recent publication, also showed a cysteine protease fold that is characteristic of the papain group of proteases. In addition, the amino acids at the catalytic site of Blo t 1 are highly conserved, as shown in Figure 1a. However, its presumed protease activity has not been experimentally demonstrated yet [28, 30]. Studies in some countries with a tropical climate have shown that Blo t 1 exhibits a high IgE reactivity. The IgE cross-reactivity between Blo t 1 and other mite cysteine proteases, such as Der f 1 and Der p 1, has been reported to be almost negligible [30, 32]. This could be due to their low sequence homology [28], as shown by multiple sequence alignments with CLUSTAL OMEGA (1.2.2) (Table 2). Recently, Meno et al. [33] also found a limited cross-reactivity between Blo t 1 and Der p 1. Future studies using well-characterized recombinant Blo t 1 should determine the importance of this allergen for *Blomia*-sensitized patients.

Blo t 2

Although 3 Blo t 2 isoallergen sequences have been deposited in the IUIS, very limited immunological data are available for the corresponding molecules. The theoretic-

cal MW of the mature chain is predicted to be 13.5 kDa for all 3 isoforms, while the theoretical pI varies from 5.9 to 6.5. Kidon et al. [11] used a slightly different sequence for their research, which achieved the expression of Blo t 2 as a fusion protein (His-tag) further purified by affinity chromatography and high-resolution size exclusion chromatography. However, rBlo t 2 showed a moderate IgE reactivity [11]. In our laboratory, we recently expressed, purified, and characterized a nonfusion recombinant version of Blo t 2 (Urrego et al., in preparation) [34]. As determined by circular dichroism and Fourier transform infrared spectroscopy analyses, the soluble rBlo t 2 showed a mixed α/β -fold with an elevated β -sheet content. Accordingly, the 3-dimensional (3-D) model of Blo t 2 based on the Der p 2 structure showed a β -barrel fold with the 2 β -sheets arranged similarly to immunoglobulin-like proteins, creating a potential ligand-binding internal cavity (Fig. 1b). ELISA and mediator release assays showed that rBlo t 2 is recognized by 54% of Brazilian patients sensitized to *B. tropicalis* [34]. Cross-inhibition assay revealed that Blo t 2 inhibited less than 40% of IgE binding to Der p 2 [34]. Therefore, rBlo t 2 can be considered as a major allergen of *B. tropicalis* and should be included in molecule-based diagnostic tests and vaccines against *Blomia* allergy.

Blo t 3

The Blo t 3 cDNA sequence was used for its recombinant production in *Escherichia coli* as a glutathione S-transferase (GST) fusion protein [35–37]. Blo t 3 has a typical globular fold (Fig. 1c) with 54.8% amino acid identity with Der p 3 (Table 2), a trypsin-like serine protease identified as an allergen in *Dermatophagoides pteronyssinus*. Furthermore, the essential residues in the active site for substrate recognition are highly conserved.

A study conducted in Singapore showed that 50% of mite-allergic patients displayed IgE reactivity to recombinant Blo t 3 [35]; however, the IgE titers were considered low. This could have been the result of improper folding of the recombinant allergen, since in that study the Blo t 3 gene was expressed without its prepro regions. It is known that these proteases when expressed as zymogens have correct folding in addition to self-inhibition enzymatic activity [38, 39]. Similarly, 57% of *B. tropicalis*-sensitized patients recognized purified natural Blo t 3, as determined by ELISA [37], but it is not clear if the purified allergen had proper folding since no data on the secondary structure was given in that work. Interestingly, a very low IgE reactivity to rBlo t 3 was reported in another study in Singapore, but no details regarding the

expressed sequence gene were presented [11]. Thus, conclusive data regarding the allergenic potential of Blo t 3 are still lacking.

Blo t 4

Sequence alignments of the Blo t 4 cDNA revealed that the allergen shares 68 and 65% sequence homology with Der p 4 and Eur m 4, respectively [29]. Blo t 4 belongs to the α -amylase family of proteins and shows the characteristic fold of β -strands surrounded by α -helices, as well as the active site (Fig. 1d). In most studies, the recombinant production of Blo t 4 has been conducted in *Pichia pastoris* [29, 40, 41]. A few studies have shown that rBlo t 4 is able to bind IgE from allergic, asthmatic, and eczema patients [11, 29, 40]; however, detailed molecule-based studies are still needed to define the clinical relevance of this allergen.

Blo t 5

Among *Blomia* allergens, Blo t 5 is by far the most studied and best characterized allergen [42–45]. Several studies reporting on the recombinant production of Blo t 5 [12, 22, 23, 40, 42–44] have shown that the allergen is recognized by IgE from 12–98% of mite-allergic and/or asthmatic patients in tropical regions of the globe [11–13, 41]. Similar to Der p 5, its counterpart in *D. pteronyssinus*, Blo t 5 has distinctive sequence characteristics: (i) 15.4% of positively charged residues, (ii) 22.2% of negatively charged residues, and (iii) low extinction coefficients due to the absence of tryptophan residues [46]. As reported for Der p 5, circular dichroism analyses showed that rBlo t 5 also presents a predominant α -helical structure [42, 44]. So far, only 1 study reporting on T-cell epitope mapping of Blo t 5 identified residues 55–70 and 58–73 as regions involved in T-cell recognition and proliferation [47]. These residues are highlighted in the Blo t 5 3-D structure depicted in Figure 1e, which also shows the flexible N-terminal region and the 3 α -helices, organized in an antiparallel manner to form a coiled-coil helical structure. While the Blo t 5 structure was determined to be monomeric, the structure of Der p 5 is dimeric, which means it has a large hydrophobic cavity that may be a ligand-binding site [48]. These cavities in allergens are normally known to bind hydrophobic ligands, which can influence the innate immune system as well as IgE-mediated diseases [48, 49]. To predict if other group 5 mite allergens could form dimers like Der p 5, Khemili et al. [50] performed an in silico analysis using MODELLER and the ClusPro server [50–52]. Interestingly, only Der f 5 was predicted to form dimers [50].

Despite sequence similarities, a very low IgE cross-reactivity between Der p 5 and Blo t 5 has been reported in several studies [42–44]. The molecular basis for the observed low cross-reactivity is unclear, as so far studies aiming to identify residues involved in IgE recognition of Blo t 5 have produced conflicting data [42, 44]. According to a study published by Chan et al. [42], Glu-76, Asp-81, Glu-86, and Glu-91 are key residues for IgE recognition of Blo t 5. In contrast, Naik et al. [44] reported that Blo t 5 residues Asn46, Lys47, Lys54, and Arg57 are involved in the formation of IgE epitopes. Figure 1e shows the identified B cell epitopes on the Blo t 5 structure. Recent studies have also evaluated the cross-reactivity between rBlo t 5 and the group 21 allergens, as well as with crude extracts of *B. tropicalis* and *Ascaris lumbricoides* [12, 53]. The latter allergen source should be considered in tropical regions due to the high prevalence of helminth infections [54–56], which could possibly interfere with the serodiagnosis of mite allergy [57, 58]. Interestingly, Carvalho et al. [12] showed that rBlo t 5 and rBlo t 21 are less cross-reactive with *A. lumbricoides* crude extracts than with extracts of *B. tropicalis*, suggesting that these 2 recombinant allergens might represent markers for the diagnosis of *B. tropicalis*-sensitized patients.

More recently, 5 polymorphic variants of Blo t 5 were identified with amino acid exchanges within IgE-binding epitopes, suggesting a variability of its allergenic properties [59]. Further studies should address the allergenicity of Blo t 5 variants as well as of other *Blomia* isoallergens.

Blo t 6

Sequence analyses have shown that Blo t 6 belongs to the chymotrypsin group of proteases. The 275-amino-acid-long sequence contains a putative signal peptide and a propeptide resulting in a predicted mature protein of 25 kDa [46]. The protein contains a peptidase S1 domain, suggesting a serine type endopeptidase activity (www.uniprot.org). The molecular modeling of Blo t 6 and the catalytic triad are shown in Figure 1f. The mature chain of Blo t 6 shares a 66% sequence homology with Der p 6 (Table 2) and both allergens contain 3 conserved disulfide bonds but lack the 4th disulfide bridge, which is typical for vertebrate chymotrypsins [46]. Although previous studies showed only a minor importance of Blo t 6 as an allergen [41], a study by Shek et al. [40] revealed that rBlo t 6 produced in *P. pastoris* is recognized by 63.3 and 76.7% of children with asthma and eczema, respectively [46]. No details were given by Shek et al. [40] regarding the Blo t 6 gene sequence, and we cannot be certain if the allergen was expressed as a zymogen, leading to a proper

folder and an influence on these higher IgE reactivity results. Thus, it is possible that the importance of Blo t 6 and the role of its propeptide are currently underestimated and a more detailed characterization is warranted.

Blo t 8

The group 8 allergen (GST) of *B. tropicalis* was identified in 1996 by screening of a *B. tropicalis* cDNA library and it was later produced as a recombinant protein [60–62]. While Blo t 8 has been shown to be clinically relevant in Colombia [60, 62], in the USA and Singapore the IgE reactivity to this allergen has been reported to be very low [11, 61]. Structural models of Blo t 8, and of Asc l 13, the GST of *A. lumbricoides*, revealed several patches of conserved and surface-exposed residues, suggesting the existence of cross-reactive and species-specific epitopes [60]. nBlo t 8 induced hypersensitivity in some patients who did not react to Asc l 13 [60], thus supporting the notion that cross-reactive and species-specific epitopes may be involved in the IgE response to GST. Figure 1g shows the structural model of Blo t 8.

Blo t 10

As a member of the tropomyosin pan-allergen family, Blo t 10 has been mostly studied regarding its cross-reactivity with other homologous allergens. In fact, the first study on a recombinant Blo t 10 aimed to clone this allergen and identify the IgE epitopes, as well as to analyze the cross-reactivity between those epitopes with Der p 10. Most of the IgE epitopes identified in Blo t 10 were located in the N- and C-terminal regions. Despite high levels of cross-reactivity between Blo t 10 and Der p 10, unique IgE epitopes of Blo t 10 were mainly found at the C-terminus of the protein [63]. Some studies have also observed cross-reactivity between rBlo t 10 and crude extracts of *A. lumbricoides* [57, 64]. In addition, rBlo t 10 was shown to inhibit the IgE-binding to a 40-kDa protein in *A. lumbricoides* extract by 85%. LC-MS/MS analyses revealed that this *A. lumbricoides* protein was a tropomyosin (Asc l 3) [57]. In addition, the similar tropomyosins rAsc l 3, rBlo t 10, and rDer p 10 were associated with asthma in another study [64]. Although the 3-D structure of mite tropomyosins has not been elucidated, a structural model of Blo t 10 is depicted in Figure 1h.

Blo t 11

Paramyosin is distributed in many invertebrates including annelids, crustaceans, nematodes, and insects. It represents a myofibrillar protein that is usually organized

in the core structure of invertebrate muscles' thick filament and interacts with myosin molecules, maintaining tension at a relatively low energy cost [65]. It has been proposed that parasite paramyosins not only serve as structural components but also act in a multifunctional manner in the host by inhibiting complement activity through binding to C1q, a component of the C1 complex [66]. Blo t 11, the paramyosin homolog in *B. tropicalis*, constitutes a high-MW allergen of around 102 kDa and an IgE reactivity >50% among asthmatic patients. The α -helical coiled coil secondary structure and the positioning of mostly apolar amino acids at positions *a* and *d* of the heptad are crucial for the formation of paramyosin dimers [67]. Although the tertiary structure of Blo t 11 has not been determined yet, the extensive coiled coil regions are also expected in its structure (Fig. 1h). Blo t 11 shows 90% sequence homology to Der f 11, the paramyosin from *D. farinae* [67], as well as 89% sequence homology to Der p 11 (Table 2). Natural and recombinant Blo t 11 are both highly susceptible to degradation, resulting in a major peptide of 66 kDa [68]. Epitope mapping with overlapping peptides revealed a dominant IgE-binding site encompassing residues 336–557 and a dominant IgG-binding site between residues 698 and 875. A synthetic peptide consisting of the IgE-binding epitope showed a higher IgE reactivity compared to recombinant full-length Blo t 11. However, it is still unclear whether sensitization is initiated against intact Blo t 11 or degraded fragments thereof [69].

Blo t 12

Formerly named Bt11a by Puerta et al. [70], Blo t 12 represents a chitin-binding protein in *B. tropicalis* showing an IgE reactivity of up to 50% with sera from mite-allergic patients [26]. Its chitin-binding activity was experimentally confirmed by Zakzuk et al. [26]. Interestingly, chitin binding has been predicted for Der p 23 and Der p 18, but only a weak binding activity has been demonstrated for Der p 18 [71–73]. The Blo t 12 sequence contains a putative signal peptide (amino acid residues 1–20) resulting in a 14-kDa mature protein [70]. Two Blo t 12 isoforms have been described so far: Blo t 12.0101, isolated from mites collected in Cartagena, Colombia, and Blo t 12.0102, isolated from mites collected in Singapore. However, only 1 isoform is listed in the IUIS allergen database. Blo t 12.0101 was claimed to represent the main isoform in Colombia but patients' reactivity to the molecule may be dependent on the geographical area [6]. Competitive inhibition ELISA with 10 overlapping synthetic peptides and sera from *B. tropicalis*-allergic pa-

tients revealed the presence of 3 linear regions that are involved in IgE binding. The most immunoreactive epitope encompasses the amino acid residues Tyr-121, Ile-122, and Thr-123. The authors suggested that the identified linear stretches are segments of larger conformational epitopes [6]. The 3-D structure of the 69-amino acid chitin-binding domain of Blo t 12 has been elucidated in solution by nuclear magnetic resonance (NMR) (Fig. 1i). As this structure has not been yet published, the data can be presently accessed in the PDB database under the code 2MFK (<http://www.rcsb.org/pdb>).

Blo t 13

Similarly to Der p 13, Blo t 13 also constitutes a minor allergen, being recognized by 11% of *B. tropicalis*-allergic patients [74]. The sequence was isolated from a cDNA library and the encoded molecule identified as a fatty-acid-specific fatty-acid-binding protein, which belongs to the cytosolic lipid transfer protein family. Lipid-binding assays experimentally confirmed the fatty-acid-binding properties of rBlo t 13, which can bind *cis*-parinaric acid and oleic acid [27]. Predictive analysis has shown the absence of glycosylation sites in the mature 14.8-kDa allergen [27, 74]. Molecular modeling of Blo t 13 predicts an architecture consisting of 10 antiparallel β -strands forming 2 β -sheets surrounding an internal pocket or barrel structure and 2 short α -helices positioned at the end of the barrel (Fig. 1j) [27, 75]. Mouse anti-rBlo t 13 IgG1 monoclonal antibodies were generated using partially purified rBlo t 13 produced in *P. pastoris*. Inhibition experiments suggested the presence a Blo t 13-homologous allergen in extracts of the mite *D. siboney* [76]. Further inhibition studies with other species could provide deeper insights into the cross-reactivity patterns of fatty-acid-binding proteins.

Blo t 19

Very little is known about *B. tropicalis* Blo t 19, a 7-kDa minor allergen showing homology to an antimicrobial peptide [77]. In the IUIS entry (www.allergen.org), the IgE-reactivity of Blo t 19 was reported by Thomas et al. (pers. commun.). A review article published in 2001 stated that 10% of allergic patients recognized recombinant Blo t 19 [77]. Nevertheless, the 3-D structure of Blo t 19 has been elucidated in solution by NMR spectroscopy and the data deposited in the PDB database under the code 2MFJ (<http://www.rcsb.org/pdb>). Similar to Blo t 12, the structure has not been published yet. This low MW allergen consists of 1 chain forming a single α -helix and 2 anti-parallel β -sheets (Fig. 1k).

Blo t 21

The major *B. tropicalis* allergen Blo t 21 was identified and characterized in 2007 by Gao et al. [78]. Although the physiological function of Blo t 21 has not been elucidated yet, the 13-kDa protein is predominantly localized in the midgut and hindgut of the mite and can also be detected in fecal particles [78]. As Blo t 21 shares over 40% sequence identity with Blo t 5 (Table 2) and the structures of both proteins resemble each other (Fig. 1e, l), it has been hypothesized that Blo t 21 is a paralog of group 5 mite allergens that originated by gene duplication [79]. Despite their similarities, the allergens are not highly cross-reactive. In fact, compared to Blo t 5, Blo t 21 displays dissimilar arrangements of charged residues on the surface. Furthermore, the cross-reactivity to other group 21 mite allergens (e.g., Der f 21) is low to moderate. Interestingly, a high percentage of *B. tropicalis*-allergic patients show sensitization to Blo t 5 and 21 [12, 78]. The 3-D structure revealed in solution by NMR spectroscopy showed that Blo t 21 is a typical α -helical protein consisting of 3 antiparallel α -helices arranged in an elongated helical bundle (Fig. 1l) [79]. The protein contains a high percentage of surface-exposed charged residues (35.4%), with basic and acidic residues being evenly distributed. Site-directed mutagenesis of several surface-exposed charged residues was used to identify the amino acids involved in IgE-binding. In this way, residues Glu-74, Asp-79, Glu-84, Glu-89, and Asp-96, respectively, were shown to form distinct patches of IgE-binding residues located in opposite sides of the allergen. The Lys-107 residue has also been proposed to be involved in IgE recognition of Blo t 21 [79] (Fig. 1l).

Posttranslational Modifications of *B. tropicalis* Allergens

The choice of host for the recombinant production of allergens has important implications when considering posttranslational modifications. Usually, recombinant allergens are produced in bacterial expression systems [59, 61, 72, 79]. Although the majority of bacterium-based expression systems are able to produce biologically active and correctly folded molecules, for a few allergens a host that allows more elaborated posttranslational modifications is required [25, 67–69]. In this respect, eukaryotic expression systems are able to perform posttranslational modifications such as disulfide bond formation, processing of signal sequences, lipid modifications, and glycosylation [80]. Glycan modifications in allergens might be directly involved in antigen uptake

and presentation. Moreover, cross-reactive carbohydrate determinants have been reported to induce carbohydrate-specific IgE antibodies [81–83]. Some studies have experimentally identified glycosylation sites in natural allergens as well as in recombinant allergens, highlighting their importance for IgE binding and for their recognition by innate immune cells [82, 84–86]. For *Blomia* allergens, on the other hand, data on glycosylation have been mostly restricted to bioinformatics predictions [29, 30, 62, 67, 70, 74]. Using a glycosylated isoform of Blo t 12 as a control, Acevedo et al. [60] were not able to detect glycan groups on natural Blo t 8 [62]. No glycan groups were found in recombinant Blo t 11 expressed in baculovirus, although the molecule has 5 predicted glycosylation sites [25, 67]. No potential glycosylation sites were detected for Blo t 4 and Blo t 13 allergens [29, 74]. The predicted Blo t 1 glycosylation site was recently confirmed in the crystal structure of recombinant proBlo t 1 [30, 33]. Despite that, experimental data on glycosylation of other *Blomia* allergens as well as other posttranslational modifications are still needed. Recently, our group obtained rBlo t 2 with all of the disulfide bonds, which were confirmed by MS analysis [34]. The allergen was expressed in *E. coli* Shuffle T7, which is known to properly form disulfide bridges [87].

Diagnosis and Immunotherapy of *B. tropicalis*

Currently several biotechnological companies offer different options for allergy diagnosis in singleplex or multiplex setups. The materials used for diagnosis are either extracts prepared from the allergen source or purified natural or recombinant allergens. For singleplex diagnostic setups, Thermo Fisher ImmunoCap®, Siemens ImmuLite®, and HyCor HyTec® offer tests with *B. tropicalis* extracts for their respective systems [88–90]. For multiplex diagnostic setups, Thermo Fisher offers the ImmunoCap ISAC®, which contains rBlo t 5 on the chip [88, 91]. The Allergome partnership recently released FABER 244, a new allergy multiplex test including *B. tropicalis* extract on the microarray [92]. Thus, the commercially available systems for diagnosis of *B. tropicalis* allergy are rather limited concerning specific allergens. To distinguish between true cosensitization and cross-reactivity between *B. tropicalis* and other mites, it would be important to include more specific allergens to the common diagnostic platforms [93]. An ELISA kit for quantification of Blo t 5 in environmental samples or *Blomia* extracts is available from Indoor Biotechnologies [2, 12, 94].

Diagnostic tests based on commercially available extracts have disadvantages related to the composition and amount of individual components [95, 96]. Thus, the absence of certain allergens in extracts can contribute to mistakes in routine diagnostic procedures [95, 97]. In addition, such problems also affect the outcome of allergen-specific immunotherapy [97]. Although some studies have shown an absence of some allergens in commercially available *D. pteronyssinus* extracts [96, 97], comparative studies have not been performed for *B. tropicalis* extract-based products.

Finally, although a number of recombinant- and/or hypoallergenic molecule-based approaches have been described for therapy of allergies caused by a variety of allergen sources [93], such approaches have not yet been developed for *B. tropicalis* allergy.

Concluding Remarks

In tropical environments, allergy-eliciting dust and storage mites constitute overlapping populations. Constant high temperatures and humidity levels favor mite growth; consequently, mite allergen exposure can be high throughout the year. The most common mite species in the tropics are *B. tropicalis*, classified as a storage mite, as well as dust mites species belonging to the genus *Dermatophagoides* [9, 98]. Moreover, other *Blomia* species as well as other mites can play an important role in sensitization in this part of the globe [99–101].

Despite their common living conditions, some of these mites are distantly related, which is also reflected in patients' IgE reactivity profiles towards the different allergenic compounds. The serodominant allergens of *Dermatophagoides* species, for example, belong to groups 1, 2, and 23, while the proposed major allergens of *Blomia* are Blo t 5 and 21, respectively. In *Dermatophagoides*, group 4, 5, 7, and 21 allergens are of secondary importance, whereas *Blomia* Blo t 1 and Blo t 2 are classified as mid-tier allergens [102]. Good epidemiologic data on sensitization profiles of *B. tropicalis* are available, mainly for Blo t 5 [12, 13, 43], while sensitization data for other *Blomia* allergens including Blo t 1, 2, 10, or even 21 are relatively scarce [11]. Even in tropical climates, sensitization to Blo t 5 may vary between the 12% reported for *Blomia*-sensitized patients in Thailand and 92.8% in Brazil and even 96.7% for asthmatic patients in Singapore [12, 40, 41]. Incomplete data on the IgE reactivity of *B. tropicalis* allergens and their clinical relevance still represent an obstacle for the development

of molecule-based diagnostic and immunotherapy of *Blomia* allergy.

Studies to identify marker allergens for *Blomia* and *Dermatophagoides* sensitization are also lacking. In addition, sensitization to other *Blomia* species has not been fully explored. A few studies recently provided data on sensitization to *B. tjiobodas*, *B. kulagini*, and *B. freemani* [99, 100, 103–105]. However, data on cross-reactivity between *Blomia* species are not generally available. Until now, only 1 study has shown that extracts of *B. tropicalis* and *B. kulagini* are cross-reactive [106].

In contrast, most studies have focused on cross-reactivity between *B. tropicalis* and other mites [33, 53, 101, 107]. At least 5 cross-reactive allergens have been identified in extracts of *Euroglyphus maynei* and *B. tropicalis* [108]. *Lepidoglyphus destructor* recombinant Lep d 2 was shown to inhibit a 14.5-kDa allergen in *B. tropicalis* extracts [109]. Also, *B. tropicalis* extract was shown to inhibit 9 allergens in *Suidasia medanensis* extracts, while *S. medanensis* inhibited IgE binding to only 4 allergens in *B. tropicalis* extracts [101].

In general, most major and mid-tier allergens from *B. tropicalis* share sequence identities below 50% with *D. pteronyssinus* allergen counterparts, except for tropomyosins, which show sequence identities of up to 90% between different mite species (Table 2). Despite the low sequence identity scores, some degree of cross-reactivity between group 5 and 21 allergens of *B. tropicalis* and *Dermatophagoides* has been reported; however, while Der f 5 could inhibit 60% of IgE binding to Blo t 5, there was no inhibition in the opposite direction [53]. Similarly, the cross-reactivity between Der p 1 and Blo t 1 was shown to be moderate [32], as was that between Blo t 5 and Der p 5 [43]. However, significant cross-reactivity has been reported between the highly conserved mite tropomyosins [63]. Altogether, published data suggest that individuals living in tropical environments are at least to some extent cosensitized by the dominant mite genera *Blomia* and *Dermatophagoides*. For successful therapeutic interventions, it seems imperative to identify the disease-eliciting allergens in their respective sources and use them in component-resolved diagnosis. This, however, requires the availability of a broad panel of high-quality and standardized allergen preparations for a reliable clinical diagnosis. In this context, it has been shown that *E. coli*- and *P. pastoris*-produced Blo t 13 show significant differences in their reactivities with a panel of murine monoclonal antibodies [76]. Likewise, rBlo t 6 produced in *P. pastoris* was more reactive than its counterpart produced in *E. coli* [40, 46]. In another study, recombinant Blo t 11 produced in insect cells was

clearly outperformed in IgE-binding assays by the natural counterpart [25]. These differences in IgE reactivity could be linked to the expression system used for recombinant production. In addition, in some cases the recombinant allergens have not been fully evaluated concerning their folding, purity, identity, and aggregation status.

To date, 13 *Blomia* allergens have been officially acknowledged by the WHO/IUIS allergen nomenclature subcommittee. However, additional allergens have been described not only in *Blomia* but also in other mite species [19]. Moreover, isoforms have been described for Blo t 1, Blo t 2, and Blo t 12, which can also have distinct IgE-binding or T-cell-activating properties, as demonstrated for the Blo t 2 homolog of *D. pteronyssinus* [6, 110]. Although substantial progress has been made since the cloning of Blo t 5 in 1995, the establishment of a comprehensive panel of high-quality *Blomia* allergens is a major challenge – and opportunity – for molecular allergologists to develop better diagnostic and therapeutic tools for managing *B. tropicalis* allergy [21].

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