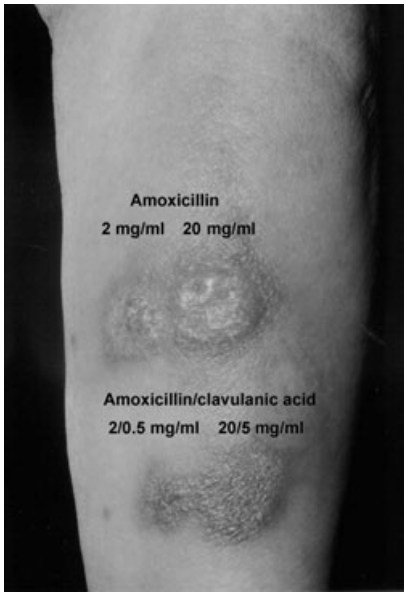

Figure 1 Positive results at 48 h of intradermal test with amoxicillin (2 and 20 mg/ml) and amoxicillin/clavulanic acid (2/0.5 and 20/5 mg/ml).

as fever, limphadenopathies or hepatomegaly to think about drug-induced hypersensitivity syndrome and as the reaction was already remitting, laboratory tests were not performed. The patient refused a challenge test to determine tolerance to alternative betalactams such as cephalosporins and refused any further studies.

Allergic reactions to betalactams are the most common cause of adverse drug reactions mediated by specific immunological mechanisms. The diagnosis of betalactam allergy is well established and can be determined using the standardised diagnostic procedures of the European Network for Drug Allergy (ENDA) (2).

Urticaria and rarely anaphylaxis (even fatal anaphylactic shock) are known to have occurred after IDT, in particular with drugs (3, 4). These unwanted side-effects occur in IgE-mediated immediate hypersensitivity reactions. Systemic reactions after skin testing for nonimmediate drug eruptions seem to be rare, although a few cases have been reported (5, 6).

This case illustrates the need for individual risk evaluation when undertaking drug allergy testing. Although in this case the clinical history was suggestive of a desquamative nonimmediate reaction to amoxicillin/clavulanic acid, which is a contraindication for performing a challenge test with the suspected drug, the positivity of skin tests is useful to confirm the clinical suspicion and to find alternative options. IDT should always be performed cautiously, as severe local reactions or systemic side-effect may occur.

The possibility of performing patch tests prior to IDT or the use of higher dilutions should be assessed, not only in patients who have suffered severe skin reactions (e.g. TEN, severe bullous exanthema, AGEP, SJS) or systemic reaction (e.g. DRESS), but also in patients who have presented a less severe but desquamative eruption, even though the sensitivity of these tests would be lower.

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Molecular cloning and immunologic characterization of For t 2: a major allergen from the biting midge *Forcipomyia taiwana*

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Keywords: allergenicity; biting midge allergy; *Forcipomyia taiwana*; immunogenicity; recombinant allergen.

This report presents the cloning and characterization of a major allergen, For t 2, from the biting midge *Forcipomyia taiwana* and demonstrates its IgE-binding capacity and ability to stimulate skin fibroblasts.

Forcipomyia taiwana (biting midge) is a tiny blood-sucking midge approximately 1–1.5 mm in size and distributed island-wide in urban and suburban Taiwan as well as in southern China (1). It is the most important cause of insect bite allergy in Taiwan. Female midges attack exposed body parts during the day, causing intense itching and swelling in sensitive individuals. About 60% of exposed subjects in Taiwan develop hypersensitivity reactions (2). A report has previously identified that the 24 (For t 1), 36 (For t 2), and 64 (For t 3) kDa proteins as major *F. taiwana* allergens (2). This study aimed to clone and characterize the 36 kDa major allergen of the midge, termed For t 2.

A ZAPII cDNA library was constructed from *F. taiwana* mRNA. It was screened with pooled sera from 50 midge-allergic subjects by plaque immunoassay. The cDNA insert of IgE-reactive clones were sequenced and subcloned into expression vectors pQE-30, transformants carrying the correct insert

fermented, and the recombinant protein purified by His-tag affinity column.

An IgE-reactive clone containing 1066 base pairs was identified. The cDNA and the deduced amino acid sequences were described elsewhere (GeneBank accession number EU678971) (3). Computer-assisted homology search revealed that the For t 2 sequence had 65–77% identity with eukaryotic translation initiation factor 3 subunit (eIF3) from many insects. The IgE reactivity of recombinant For t 2 was determined *in vivo* by skin prick test and *in vitro* by ELISA (Table 1).

Furthermore, rabbit anti-rFor t 2 antibodies were used for immunohistochemistry staining. The For t 2 protein was abundantly expressed in the salivary glands, brain, flight muscles, and midgut of the midge (Fig. 1A).

Whether or not rFor t 2 could activate skin fibroblasts, the Hs68 cells, aside from its IgE-binding capacity, was further examined. Skin fibroblasts represented cells constituting the innate immune barrier that first come in contact with biting midges and are well-known source of chemokines. The direct effect of rFor t 2 on IL-6 and IL-8 protein secretion from human skin fibroblasts was analyzed by ELISA. Hs68 cells secreted IL-6 (Fig. 1B) and IL-8 (Fig. 1C) after stimulation with rFor t 2 in a concentration-dependent manner.

The recombinant For t 2 reacted with 58–64% of midge-allergic subjects and was abundantly expressed in the biting midges, which support the possibility of this protein entering the host body

Table 1 IgE reactivity of recombinant For t 2 by skin prick test and ELISA

		Midge-allergic (n = 50)	
	Nonallergic control (n = 5)	rFor t 2 nonreactive	rFor t 2-reactive
Age	29.40 ± 7.20	38.26 ± 13.09	34.25 ± 10.67
Number of positive SPT to rFor t 2	0	0	29 (58%)
Gender (M/F)	2/3	5/16	7/22
SPT to rFor t 2-W/E (mm^2 ± mm^2/mm^2 ± mm^2)	0 ± 0/0 ± 0	0 ± 0/0 ± 0	1.39 ± 0.13/6.34 ± 0.56
Number of positive ELISA to rFor t 2	0	0	32 (64%)
Mean OD value to rFor t 2	0.12 ± 0.02	0.24 ± 0.02	1.33 ± 0.20

M, male; F, female; SPT, skin prick test; W, wheal; E, erythema; OD, optic density.

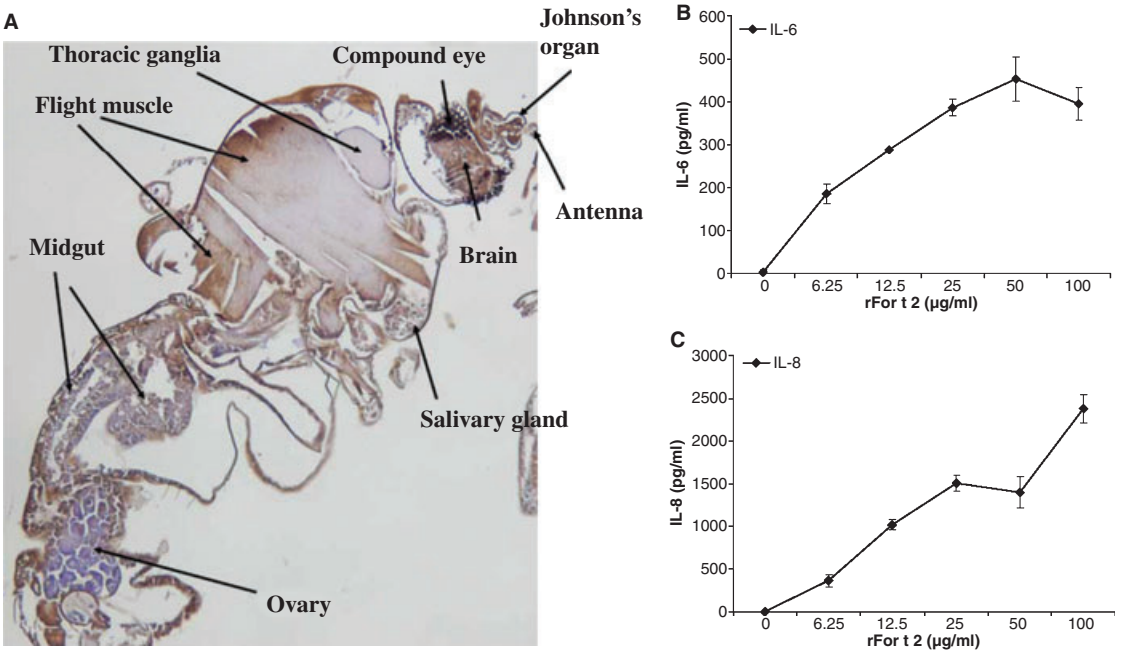

Figure 1 (A) Target proteins were stained with 3,3′diaminobenzidine for 3 min and counterstained with hematoxylin. The For t 2 proteins were abundantly expressed in the salivary glands, brain, flight muscles, and midgut in the midge (dark brown spots). rFot t 2 induced (B) IL-6 and (C) IL-8 protein secretions from human skin fibroblasts Hs68 cells. Hs68 cells were stimulated with various concentrations (6.25–100 μg/ml) of rFor t 2 for 24 h. IL-6 and IL-8 in the culture supernatant were determined by ELISA. Data were mean ± SEM of three independent experiments.

through the bite. Recombinant For t 2 had significant sequence identity with eIF3, which was required for the binding of mRNA to 40S ribosomal subunits and the interaction with many components of the translational machinery (4).

Increased mRNA and protein levels of eIF3 have been detected in a wide variety of human cancers and are frequently identified as biomarkers for poor clinical outcome (4). However, members of the eIF protein family have never been reported as allergens and therefore represent a new class of IgE-binding proteins. There has been increasing evidence that allergic inflammation involves not only the Th2-cell pathway but also innate immune responses. Many allergens are able to initiate or propagate inflammation via stimulating cell receptors constituting the physical skin barrier (5, 6). This report has shown that For t 2 not only binds with serum IgE of sensitized patients but also induces the production of important inflammatory chemokines from skin fibroblasts in a concentration-dependent manner.

In conclusion, this study has cloned a major biting midge allergen, For t 2, with significant sequence homology to eIF3, thus defining a new class of allergens that can stimulate IL-6 and IL-8 secretion from human fibroblasts. The purified recombinant allergen may be used for diagnosing and treating midge bite allergy in the future.

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Relative lack of T regulatory cells in adult eosinophilic esophagitis – no normalization after corticosteroid therapy

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Keywords: allergy; eosinophilic esophagitis; Foxp3; T cells; T regulatory cells.

Eosinophilic esophagitis (EoE) is a chronic-inflammatory, Th2-type allergic disease of the esophagus with a rapidly increasing prevalence. EoE is clinicopathologically characterized by symptoms attributed to esophageal dysfunction in combination with a dense esophageal eosinophilia, both of which are refractory to proton pump inhibitors (1). T regulatory cells represent a subset of T cells that play a central role in the prevention of immunopathology and might be decreased in either numbers or function in autoimmune and allergic diseases (2). Foxp3 represents a crucial transcription factor required for the large majority of T regulatory cells (2).

In active eosinophilic esophagitis, the proportion of Foxp3 positive T cells is reduced by about 50%. The relative lack of T regulatory cells in this allergic disorder is not reversible by corticosteroid therapy, which is otherwise successful in bringing patients in clinical and histological remission.

We have recently reported that budesonide, in a clinical, randomized, double-blinded, placebo-controlled trial, significantly reduced both clinical symptoms and mean eosinophil numbers in the esophageal epithelial layer of adult EoE patients (3). Here, we report the results of T regulatory cell number measurements, which we additionally analyzed within this study (EoE patients, $n = 35$). As control group, we recruited 20 esophagus-healthy individuals. In the epithelium of the esophagus, numbers of total T cells and T regulatory cells were assessed by immunofluorescence and confocal microscopy. Indirect immunostainings were performed on four proximal and four distal biopsies of each patient and control individual, respectively, as previously described (3). Rabbit anti-CD3 (DakoCytomation, Glostrup, Denmark) and monoclonal anti-Foxp3 (clone PCH101; eBioscience, Frankfurt, Germany) were used as primary antibodies. Representative examples of the original immunofluorescence data are shown in Fig. 1A. Positive cells were counted in a blinded manner in 20 hpf (area of 1 hpf 0.0538 mm^2) to obtain average numbers per 1 hpf.