

Mohamed Badawy Abdel-Naser · Ranya Adel Lotfy ·
Maged Mustafa Al-Sherbiny ·
Nehad Mahmoud Sayed Ali

Patients with papular urticaria have IgG antibodies to bedbug (*Cimex lectularius*) antigens

Received: 26 August 2005 / Accepted: 27 September 2005 / Published online: 20 January 2006
© Springer-Verlag 2006

Abstract IgG and IgE against salivary gland proteins of bedbug (*Cimex lectularius*) were assessed in comparison with mosquito (*Culex pipiens*) and flea (*Pulex irritans*) antigens in the sera of papular urticaria patients (group I), siblings without papular urticaria (group IIa), patients' parents (group IIb), and healthy controls (group III) (Immunoblotting). Anti-*C. lectularius* IgG was significantly recognized at 66 and 10 kDa in 40% of group I, besides others ranging from 45 to 107 kDa. Group IIa significantly reacted with 70 kDa (57.1%). Group IIb reacted with 21 and 8.5 kDa (26.7%). Sixty percent of group IIb and 100% of group III significantly identified a band of 12.5 kDa. IgG against *C. pipiens* was significantly recognized at a range of 18–105 kDa in group I, IIb (115, 7 kDa), and III (58, 50 kDa). Anti-*P. irritans* IgG was significantly recognized by group I (100, 70 kDa) and group IIa (60, 35 kDa). IgE response was confined to *C. pipiens* at 115 and 54 kDa in groups I and III, respectively, besides 68 and 58 kDa in group IIa. It is concluded that IgG is present against *C. lectularius*, *C. pipiens*, and *P. irritans* in papular urticaria and may contribute to its pathogenesis.

Introduction

Papular urticaria is a common, chronic or recurrent eruption of pruritic papules, often grouped in irregular clusters,

M. B. Abdel-Naser · R. A. Lotfy
Department of Dermatology and Venereology,
Faculty of Medicine, Ain Shams University,
Cairo, Egypt

M. M. Al-Sherbiny
Department of Zoology, Faculty of Science,
Cairo University,
Cairo, Egypt

N. M. Sayed Ali (✉)
Department of Parasitology, Faculty of Medicine,
Ain Shams University,
11566, Cairo, Egypt
e-mail: nehad1112@yahoo.com
Tel.: +20-26704392

frequently seasonal in incidence, and predominantly affects children. It is generally regarded as a hypersensitivity or id reaction to bites from insects mostly mosquitoes, fleas, and bedbugs (Stibich and Schwartz 2003).

Several attempts to characterize human IgG and IgE antibody response to mosquitoes were made using either whole body or salivary gland extracts (Wu and Lan 1989; Konishi 1990; Das et al. 1991). Shen et al. (1989) found IgG- and IgE-binding antigens in *Aedes albopictus* mosquito whole body extract and Penneys et al. (1989) detected IgG antibodies against salivary glands' proteins from different mosquito species. Similarly, a diverse group of flea proteins was recognized by IgG and IgE in patients with papular urticaria (Garcia et al. 2004).

Bedbug bites can create considerable anxiety and localized and occasionally systemic reactions. Papular urticaria, extensive erythemas, urticaria, and even anaphylaxis were reported with the bites (Stibich et al. 2001). Although antibodies were documented and well-characterized against mosquitoes and fleas, little is known about the presence of antibodies against bedbugs. This study aimed at comparing IgG and IgE antibodies responses in papular urticaria patients against salivary gland extracts of bedbugs (*Cimex lectularius*), mosquitoes (*Culex pipiens*) and fleas (*Pulex irritans*) using immunoblotting.

Materials and methods

Patients and control

IgG and IgE antibodies against *C. lectularius*, *C. pipiens*, and *P. irritans* were studied in the whole serum of papular urticaria patients ($n=15$; age range, 6 months to 6 years; mean age, 2.42 ± 1.57 yrs group I) who had attended the outpatient clinic of Dermatology, Ain Shams University Hospital, Cairo, Egypt. Serum was also assessed in unaffected siblings ($n=7$; mean age, 3.3 ± 2.32 yrs) sharing the same environment (group IIa), their parents ($n=15$; mean age, 27.67 ± 4.34 yrs) exposed to same environmental conditions, and those without papular urticaria (group IIb). The

negative controls (group III) included healthy individuals ($n=10$; mean age, 2.6 ± 1.76 yrs). All the participants or their guardians gave written informed consent before joining the study.

Preparation of bedbug (*C. lectularius*) and flea (*P. irritans*) extracts

Bedbugs (*C. lectularius*) and fleas (*P. irritans*) were collected alive, killed separately by immersion in ether, and dissected in the head and salivary glands under a binocular microscope. Complete drying of head and salivary gland extracts of *P. irritans* and *C. lectularius* was achieved by placing them in an oven at 40°C for 1–2 days. This was followed by grinding of the dried extracts. Then 1 g of each of the insect's extract was mixed with nine parts (w/v) of Coca's solution (5 g NaCl, 2.75 g NaHCO_3 , and 4 g phenol/l distilled water). Shaking the insect extract solutions well for 2 h daily for three successive days followed by primary filtration using Büchner's funnel were done. Secondary filtration of the extract solutions by Sietz filter was also done. The antigens were aliquotted and stored at -70°C (Schaffer 1959). The protein concentration of the *C. lectularius* antigen preparation was 1 mg/ml while that of *P. irritans* was 0.570 mg/ml as measured using the Bradford method (Bradford 1976).

Preparation of mosquito (*C. pipiens*) extract

Female mosquitoes (*C. pipiens*) collected alive were anesthetized by chilling them at 4°C in a refrigerator. Salivary glands were dissected from female *C. pipiens* in 0.02 M phosphate-buffered saline (PBS) at pH 7.2 under a binocular microscope and were immediately transferred to 1 ml of PBS on ice, ultrasonicated for 30 s, and centrifuged at $8,820\times g$ for 15 min. The supernatant was collected, aliquotted, and stored at -70°C (Peng et al. 1996). The protein concentration of the antigen preparation was 2 mg/ml as measured using the Bradford method (Bradford 1976).

SDS-PAGE and immunoblotting

C. lectularius, *C. pipiens*, and *P. irritans* proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing condition (at a concentration of $2\ \mu\text{g}\ \mu\text{l}^{-1}\ \text{mm}^{-1}$) on a 12% gel as previously described (Laemmli 1970). The separated proteins were transferred to $0.45\ \mu\text{m}$ nitrocellulose papers (NCPs) (BioRad). The NCPs for each antigen were cut into 4 mm strips and blocked in PBS containing 0.3% Tween 20 (0.3% PBST) solution with 5% nonfat milk.

IgG immunoblotting Strips of each antigen were incubated with diluted sera in 0.3% PBST (1:50 for *C. lectularius* and 1:25 for *P. irritans* and *C. pipiens* antigens) for 1 h at

room temperature. After washing with 0.3% PBST, the strips were incubated with peroxidase conjugated goat anti-human IgG (1:1,000 in 0.3% PBST), washed, and incubated with 0.02% diaminobenzidine substrate (DAB) (Sigma, CA, USA) and 0.01% H_2O_2 for 15 min. The reaction was stopped by distilled water (Tsang et al. 1983).

IgE immunoblotting Strips of each antigen were incubated with diluted sera in 0.05% PBST (1:25) for 2 h at 37°C . After washing with 0.05% PBST, the strips were incubated with mouse anti-human IgE labeled with biotin (PharMingen, USA) (1:1,000 in 0.05% PBST) for 2 h at 37°C . After washing, the strips were incubated with streptavidin peroxidase (PharMingen) (1:1,000 in 0.05% PBST) for 1 h at 37°C , washed, and incubated with 0.02% DAB and 0.01% H_2O_2 for 15 min at 37°C . The reaction was stopped by distilled water (Tsang et al. 1983).

Statistical analysis

Analysis of data was performed using Statistical Package for the Social Sciences (SPSS) 8 for Windows. Descriptive statistics was done for qualitative data by number and percent. Pearson chi-square was done for comparison between nonnumerical data. The p values <0.05 were considered significant.

Results

Antibodies to bedbug (*C. lectularius*) proteins

IgG Several proteins were recognized in all groups. Only the patients' sera (group I) significantly had IgG antibodies against 66 and 10 kDa (group I vs III, $p<0.05$; IIb $p<0.01$; IIa, $p>0.05$). Also, all sera (100%) significantly reacted with 98–107 and 54–56 kDa (group I vs IIb and III, $p<0.001$; IIa, $p<0.05$), 45 kDa (group I vs IIb and III, $p<0.001$), and 37 kDa (group I vs IIb, $p<0.01$; IIa, $p<0.05$; III, $p>0.05$). Lower but significant reactivity was observed with 79 kDa (26.7%; group I vs IIb, $p<0.05$; IIa and III, $p>0.05$) and 64 kDa (60%; group I vs III, $p<0.01$; IIb, $p<0.001$; IIa, $p>0.05$).

A band of 70 kDa was significantly recognized in group IIa (group IIa vs I and III, $p<0.01$; IIb, $p>0.05$). On the other hand, the sera of groups IIb and III, but not group I, significantly showed a band of 53, 40 kDa (group IIb vs I, $p<0.05$) and 12.5 kDa ($p<0.001$). Bands of 21 and 8.5 kDa were only recognized by group IIb (group IIb vs I, $p<0.05$; IIa and III, $p>0.05$) (Figs. 1 and 2, Table 1).

IgE When *C. lectularius* antigen was probed with the sera of all groups of the study, a band of 22 kDa molecular weights (MWs) was detected by the sera of 13 patients (86.7%), six sera of group IIa (85.7%), 14 sera of group IIb (93.3%), and all sera of group III (100%). Another band of 37 kDa was detected by one serum of group IIb. There was no significant statistical difference for these bands.

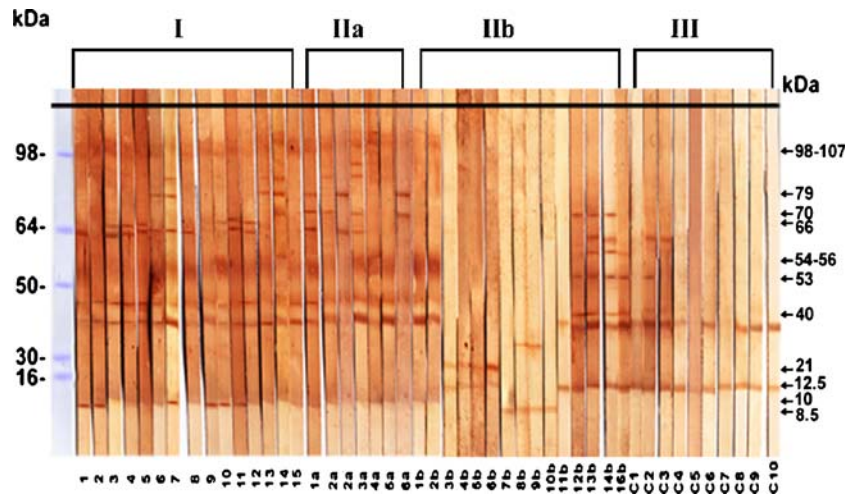


Fig. 1 Immunoblot analysis of IgG response to *Cimex lectularius* extract in the different groups of the study (I, IIa, IIb and III). Arrows point to significant reactions at 66 and 10 kDa that were detected by group I only (group I vs III, $p < 0.05$; group I vs IIb, $p < 0.01$; IIa, $p > 0.05$), 8.5 and 21 kDa by group IIb only (group IIb vs

I, $p < 0.05$; IIa and III, $p > 0.05$), 40 and 53 kDa by group IIb ($p < 0.05$), 12.5 kDa by groups IIb and III ($p < 0.001$), 98–107 and 54–56 kDa by group I (group I vs IIb and III, $p < 0.001$; IIa, $p < 0.05$), 79 kDa also by group I ($p < 0.05$), and 70 kDa by group IIa (group IIa vs I and III, $p < 0.01$; IIb, $p > 0.05$)

Antibodies to mosquito (*C. pipiens*) proteins

IgG Sera of group I significantly recognized 105, 96, 85, 37, and 18 kDa when compared with group IIb and the control, i.e., group III ($p < 0.05$ – 0.001), but not group IIa ($p > 0.05$). Although a band of 60 kDa was recognized by 20% of the sera of group I, it was also recognized by 60% of the sera of group III ($p < 0.05$), though the sera of group I significantly recognized bands of 54 kDa (group I vs IIb, $p < 0.001$), 80, and 52 kDa (group I vs IIb, $p < 0.05$). Other bands recognized by the sera of group I, e.g., 76, 70, 62, and 33 kDa, were not significant ($p > 0.05$).

Sixty percent of group III sera significantly reacted with the band of 58 kDa (group III vs I and IIb, $p < 0.01$; IIa, $p < 0.05$) and all sera exclusively reacted with 50 kDa (group III vs other groups, $p < 0.001$). Furthermore, a band of 115 kDa was significantly recognized by 60% of the

sera of group IIb (group IIb vs I, $p < 0.001$; III, $p < 0.01$; IIa, $p < 0.05$). Another band of 7 kDa was significantly recognized only by 46.7% of the sera of group IIb (group IIb vs I, $p < 0.01$; III and IIa, $p < 0.05$). On the other hand, the sera of group IIa recognized nearly the same bands as group I with different percentages. Only a band of 54 kDa, which was detected by both groups, had a significant statistical difference ($p < 0.05$) (Table 2).

IgE *C. pipiens* proteins were significantly reactive to IgE antibodies in the sera of group I at 115 and 82 kDa (group I vs IIb and III, $p < 0.05$ – 0.01 ; IIa, $p > 0.05$). Bands of 36–38 and 16 kDa were recognized by the sera of all groups of the study with different reactivity, however, significant statistical difference was found between groups I and III for bands 36–38 kDa ($p < 0.01$), groups I and III ($p < 0.05$) and groups I and IIb ($p < 0.01$) for the band of 16 kDa.

Fig. 2 Significant reactive proteins in *Cimex lectularius* extract to IgG antibodies in the different groups of the study (I, IIa, IIb and III)

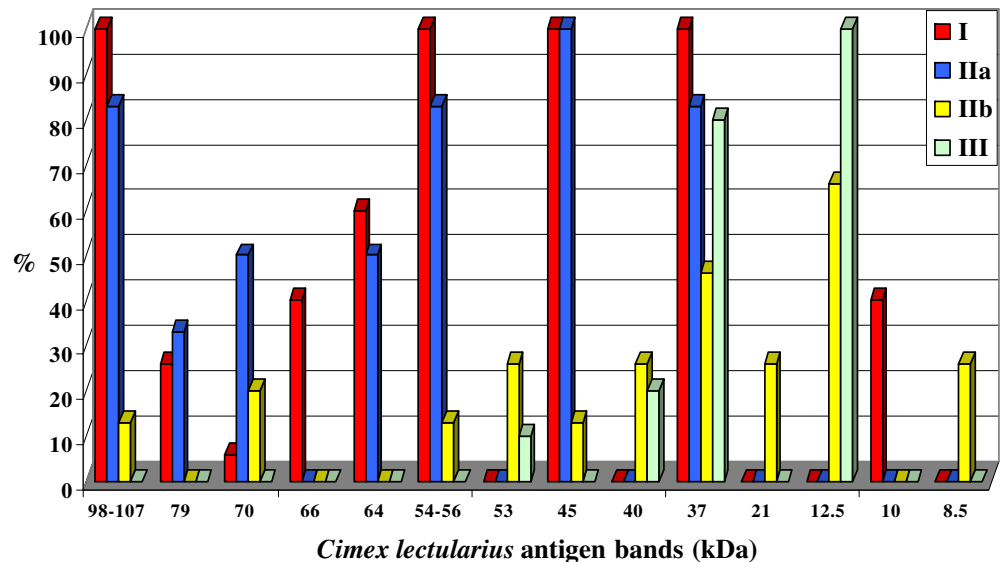


Table 1 Immunoblot analysis of IgG response against bedbug (*Cimex lectularius*) extracts

Groups	Sera (n)	Antibody prevalence no. (%positive) for each antigen band																		
		Molecular weight (kDa)																		
		98-107	91	87	79	70	66	64	60	58	54-56	53	45	40	37	30	21	12.5	10	8.5
Groups I & III	15	15***	0	3	4	1	6*	9**	0	0	15***	0	15***	0	15	0	0	0	6*	0
		(100)		(20)	(26.7)	(6.7)	(40)	(60)			(100)		(100)		(100)				(40)	
	10	0	0	0	0	0	0	0	2	0	0	1	0	2	8	0	0	10***	0	0
									(20)			(10)		(20)	(80)			(100)		
Groups I & IIa	15	15*	0	3	4	1	6	9	0	0	15*	0	15	0	15*	0	0	0	6	0
		(100)		(20)	(26.7)	(6.7)	(40)	(60)			(100)		(100)		(100)				(40)	
	7	5	1	2	3	4**	0	3	0	0	5	0	7	0	5	0	0	0	0	0
		(71.4)	(14.2)	(28.5)	(42.8)	(57.1)		(42.8)			(71.4)		(100)		(71.4)					
Groups I & IIb	15	15***	0	3	4*	1	6**	9***	0	0	15***	0	15***	0	15**	0	0	0	6**	0
		(100)		(20)	(26.7)	(6.7)	(40)	(60)			(100)		(100)		(100)				(40)	
	15	2	0	0	0	3	0	0	2	3	2	4*	2	4*	7	2	4*	9***	0	4*
		(13.3)				(20)			(13.3)	(20)	(13.3)	(26.7)	(13.3)	(26.7)	(46.7)	(13.3)	(26.7)	(60)		(26.7)

* $p < 0.05$
 ** $p < 0.01$
 *** $p < 0.001$

Table 2 Immunoblot analysis of IgG response against mosquito (*Culex pipiens*) extract

Groups	Sera (n)	Antibody prevalence no. (%positive) for each antigen band																		
		Molecular weight (kDa)																		
		115	105	96	85	80	76	70	62	60	58	56	54	52	50	37	33	18	9	7
Groups I & III	I 15	0	8**	13**	13*	12	2	3	3	3*	0	5*	15	13	0	13***	2	14***	0	0
	III 10	0	(53.3)	(86.7)	(86.7)	(80)	(13.3)	(20)	(20)	(20)	(20)	(33.3)	(100)	(86.7)	0	(86.7)	(1.33)	(93.3)	0	0
			0	3	5	10	4	2	0	6	6**	0	10	10	10***	0	0	0	0	0
			(30)	(50)	(100)	(40)	(20)	(20)	(60)	(60)	(60)	(60)	10	(100)	(100)					
Groups I & IIa	I 15	0	8	13	13	12	2	3	3	3	0	5	15*	13	0	13	2	14	0	0
	IIa 7	0	(53.3)	(86.7)	(86.7)	(80)	(13.3)	(20)	(20)	(20)	(20)	(33.3)	(100)	(86.7)	0	(86.7)	(13.3)	(93.3)	0	0
			2	5	4	6	3	0	1	0	0	0	5	5	0	7	0	5	0	0
			(28.5)	(71.4)	(57.1)	(85.7)	(42.8)	(14.2)	(14.2)	(14.2)	(14.2)	(71.4)	(71.4)	(71.4)	0	(100)	0	(71.4)	0	0
Groups I & IIb	I 15	0	8**	13***	13***	12*	2	3	3	3	0	5*	15***	13*	0	13**	2	14**	0	0
	IIb 15	9***	(530.3)	(86.7)	(86.7)	(80)	(13.3)	(20)	(20)	(20)	(20)	(33.3)	(100)	(86.7)	0	(86.7)	(13.3)	(93.3)	0	0
			0	3	3	6	0	4	0	0	0	0	6	7	0	5	0	06	2	7**
			(60)	(20)	(20)	(40)	(26.7)	(26.7)	(26.7)	(26.7)	(26.7)	(33.3)	(40)	(46.7)	0	(33.3)	0	(40.3)	(13.3)	(46.7)

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

The sera of group IIa significantly recognized bands of 68 kDa (group IIa vs I, $p < 0.001$; IIb and III, $p < 0.01$) and 58 kDa (group IIa vs I, $p < 0.01$; III, $p < 0.05$; IIb, $p > 0.05$). The band of 58 kDa was also significantly recognized by group IIb (group I vs IIb, $p < 0.05$). The sera of group III recognized three bands that were not detected by the sera of other groups at 60, 54, and 30 kDa with significant statistical difference for 54 kDa (group III vs I and IIb, $p < 0.05$; IIa, $p > 0.05$).

Antibodies to flea (*P. irritans*) proteins

IgG The sera of group I significantly showed two bands of MWs, 100 kDa (group I vs IIb, $p < 0.05$) and 70 kDa (group I vs III and IIb, $p < 0.001$; IIa, $p > 0.05$). The sera of group IIa significantly recognized bands of 60 kDa (group IIa vs I and III, $p < 0.01$; IIb, $p > 0.05$) and 35 kDa (group IIa vs I and IIb, $p < 0.01$; III, $p < 0.05$).

IgE IgE antibodies were not significantly detected in the sera of group I at bands of 132, 58, and 24 kDa with 6.7%, 13.3%, and 13.3% reactivity, respectively ($p > 0.05$). A band of 24 kDa was recognized by two sera of group IIa, whereas groups IIb and III were not reactive.

Discussion

Data of the present study show that sera of papular urticaria patients contain antibodies that were reactive not only with the antigens of *C. pipiens* and *P. irritans*, but also of *C. lectularius*.

Despite being a cause of papular urticaria (Crissey 1981; Arnold et al. 1990; Steen et al. 2004), no prior attempts were made to determine the antibody response against bedbugs in patients with papular urticaria. In this study, IgG antibodies reacted against several proteins of *C. lectularius* with a range of 8.5–107 kDa. Antigens 66 and 10 kDa were significantly recognized by 40% of patients' sera but not by the sera of other groups, pointing out that they might be specific for *C. lectularius*-induced papular urticaria. On the other hand, other antigens, namely, 98–107 and 54–56 kDa were significantly recognized by all the patients' sera when compared to other groups of the study and these bands were not even recognized by any of the controls, suggesting a relationship to the pathogenesis of the disease. Bands of 87, 79, and 64 kDa were detected in the patients and group IIa but not IIb, which may reflect correlation with age.

Similar to the controversy surrounding Hymenoptera (Aalbers et al. 1983; Bousquet et al. 1987; Gloden et al. 1992), it is not known whether anti 21 and 8.5 kDa antibodies are blocking antibodies (being recognized by the sera of group IIb only); and whether the bands of 12.5 kDa (identified by all the sera of the control group and 60% of group IIb) and 70 kDa (significantly recognized by the sera of group IIa) are also blocking antibodies. Moreover, 53 and 40 kDa bands were detected in groups IIb and

III with significant difference between the patients and group IIb, but further studies are needed to answer the question if these antibodies act as blocking antibodies.

Regarding IgE antibodies against *C. lectularius*, 22 kDa protein was recognized in patients (86.7%), group IIa (85.7%), group IIb (93.3%), and the controls (100%); therefore it is likely that it is nonspecific.

Mosquito-specific IgG was previously identified in human sera and was found to consist mainly of the IgG₄ and IgG₁ subclasses, though the role of mosquito-specific IgG remains unclear (Peng et al. 1996). Besides IgG and IgE antibodies against *A. albopictus* mosquito whole body extract, reactive IgGs against several proteins in the range of 14–126 kDa in salivary glands of *Culex nigripalpus*, *Culex quinquefasciatus*, *Aedes taeniorhynchus*, *Aedes aegypti*, and *Anopheles quadrimaculatus* have been detected (Penneys et al. 1989; Shen et al. 1989). Similarly, in the present study with *C. pipiens* salivary gland extract, IgG antibodies were detected against several proteins in the range of 18–115 kDa, which is broadly similar to previous studies.

Several proteins were recognized by anti-*C. pipiens* IgG in both the patients and controls in the present work. IgG against 105, 56, 37, and 18 kDa proteins were significantly detected in the patients' sera but not in the controls. Other proteins were significantly detected in the patients compared to the controls, namely, 96, 85, and 60 kDa. This may be similar to some antigens found by Shen et al. (1989) who reported IgG antibodies against *A. albopictus* salivary gland proteins with a range of 21–78 kDa. Brummer-Korvenkontio et al. (1994) found IgG antibodies against *Aedes communis* mosquito with 36, 30, and 22 kDa saliva proteins. The 37 kDa protein in the salivary gland extract of *C. pipiens* in the present study could correspond to the 36 kDa protein present in the saliva of *A. communis* and 34 kDa of *A. albopictus* salivary gland proteins as cross-reactivity between species is known for this protein (Garcia et al. 2004). The slight variations in the molecular weights of the detected antigens may be attributed to the differences in the species of mosquitoes, the preparations of mosquito extracts, or concentrations of SDS-PAGE used. Nevertheless, it was suggested that these antibodies may be involved in the pathogenesis of immediate mosquito bite reactions (Brummer-Korvenkontio et al. 1994).

It is interesting to note that 115 and 7 kDa were only significantly recognized by group IIb and 58 and 50 kDa only by the controls suggesting that they might act as blocking antibodies (Aalbers et al. 1983; Bousquet et al. 1987; Gloden et al. 1992) and this may provide an explanation for the decreased incidence of papular urticaria among adults. On the other hand, antibodies recognizing 37 and 18 kDa antigens may be related to recent exposure to *C. pipiens* bites as they were detected in the patients and their intimate indwellers but not in the control group.

IgE was already reported to be against salivary proteins of ten worldwide mosquito species including *C. pipiens* (Peng et al. 1998). Several antigens were identified; some were similar to those of the present study, namely, 75, 36–38, and 16 kDa. Using *C. quinquefasciatus* salivary gland

extract, Malafronte et al.(2003) found IgE antibodies against 35 and 28 kDa in the sera of all patients allergic to mosquito bites and 45 and 20 kDa in some, which is similar to the 38–39 and 46 kDa that were recognized in the present work. Brummer-Korvenkontio et al. (1994) reported IgE antibodies against *A. communis* saliva of 64, 36, 30, 22 kDa proteins. This may be similar to the 36–38 kDa antigens that were found in the present work as 37 kDa protein is a species-shared protein (Peng et al. 1998; Garcia et al. 2004). An interesting result was the recognition of 82 and 75 kDa by the sera of the patients, groups IIa and IIb, but not by the controls, which may be related to recent exposure to *C. pipiens* bite.

Both IgG and IgE were also characterized to be against flea proteins (*Ctenocephalus felis*) in of papular urticaria (Garcia et al. 2004). IgG was recognized in the patients' sera at a range of 57–111 kDa, which is similar to the 100 and 70 kDa with a reactivity of 26.7 and 93.3%, respectively, in the present study. The 60 and 35 kDa were significantly reactive to IgG antibodies in the sera of group IIa but not in the patients' sera, suggesting that they are probably blocking antibodies that may prevent the development of the disease in the patients' siblings of the same age. On the other hand, IgE reactivity against *P. irritans* antigens likely has no clinical significance.

In summary, this study has demonstrated the presence of IgG antibody response for bedbug (*C. lectularius*) in addition to mosquito (*C. pipiens*) and flea (*P. irritans*) in patients with papular urticaria. IgE response in these patients was confined to *C. pipiens* extract. Further studies are recommended to elicit the role of bedbug specific antibodies in the pathogenesis of papular urticaria. Bedbugs must be put in consideration in addition to mosquitos and fleas in attempts of vaccine development against papular urticaria.

Acknowledgements The authors would like to thank Prof. Desmond J. Tobin (University of Bradford, England) for helpful discussions on this paper.

References

- Aalberse RC, Dieges PH, Knul-Bertlova V (1983) IgG₄ as a blocking antibody. *Clin Rev Allergy* 1:289–302
- Arnold HL, Odom RB, James WD (1990) Erythema and urticaria. In: Odom RB, James WD, Berger TG (eds) *Andrews' diseases of the skin: clinical dermatology*. 8th edn. Saunders, Philadelphia, pp 154–155
- Bousquet J, Fontez A, Aznar R (1987) Combination of passive and active immunization in honeybee venom immunotherapy. *J Allergy Clin Immunol* 79:947–954
- Bradford M (1976) A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principles of protein dye binding. *Anal Biochem* 72:248–254
- Brummer-Korvenkontio H, Lappalainen P, Reunala T, Palosuo T (1994) Detection of mosquito saliva-specific IgE and IgG4 antibodies by immunoblotting. *J Allergy Clin Immunol* 93:551–555
- Crissey JT (1981) Bedbugs. *Int J Dermatol* 20:411–414
- Das MK, Mishra A, Beuria MK, Dash AP (1991) Human natural antibodies to *Culex quinquefasciatus*: age-dependent occurrence. *J Am Mosq Control Assoc* 7:319–321
- Garcia E, Halpert E, Rodriguez A, Andrade R, Fiorentino S, Garcia C (2004) Immune and histopathologic examination of flea bite-induced papular urticaria. *Ann Allergy Asthma Immunol* 9:446–452
- Golden DBK, Lawrence ID, Hamilton RH (1992) Clinical correlation of the venom-specific IgG antibody level during maintenance immunotherapy. *J Allergy Clin Immunol* 90:368–393
- Konishi E (1990) Distribution of immunoglobulin G and E antibody levels to salivary gland extracts of *Aedes albopictus* (Diptera: Culicidae) in several age groups of a Japanese population. *J Med Entomol* 27:519–522
- Laemmli UK (1970) Cleavage of structural proteins during assembly of the head of bacteriophage T₄. *Nature* 227:680–685
- Malafronte RS, Calvo E, James AA, Marinotti O (2003) The major salivary gland antigens of *Culex quinquefasciatus* are D7-related proteins. *Insect Biochem Mol Biol* 33:63–71
- Peng Z, Yang M, Estelle F, Simons FER (1996) Immunologic mechanisms in mosquito allergy: correlation of skin reaction with specific IgE and IgG antibodies and lymphocyte proliferation response to mosquito antigens. *Ann Allergy Asthma Immunol* 77:238–244
- Peng Z, Li H, Simons FER (1998) Immunoblot analysis of salivary allergens in 10 mosquito species with worldwide distribution and the human IgE responses to these allergens. *J Allergy Clin Immunol* 101:498–505
- Penneys NS, Nayar JK, Bernstein H, Knight JW, Leonardi C (1989) Mosquito salivary gland antigens identified by circulating human antibodies. *Arch Dermatol* 125:219–222
- Schaffer N (1959) Studies in allergic extracts. *Ann Allergy* 17:380–387
- Shen HD, Chen CC, Chang HN, Tu WC, Han SH (1989) Human IgE and IgG antibodies to mosquito proteins detected by the immunoblot technique. *Ann Allergy* 63:143–146
- Steen CJ, Carbonaro PA, Schwartz RA (2004) Arthropods in dermatology. *J Am Acad Dermatol* 50: 819–842
- Stibich AS, Schwartz RA (2003) Papular urticaria. In: *Dermatol. eMedicine Journal*. <http://author.emedicine.com/derm/topic911.htm>. Cited 15 Aug 2003
- Stibich AS, Carbonaro PA, Schwartz RA (2001) Insect bite reactions: an update. *Dermatology* 202:193–197
- Tsang VCW, Peralta JM, Simons AR (1983) Enzyme-linked immunoelectrotransfer blot techniques (EITB) for studying the specificities of antigens and antibodies separated by gel electrophoresis. *Methods Enzymol* 92:377–391
- Wu CH, Lan JL (1989) Immunoblot analysis of allergens in crude mosquito extracts. *Int Arch Allergy Appl Immunol* 90:271–273